A SURVEY OF THE SYMBIOTES FOUND IN ANIMAL EMBRYOS

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A SURVEY OF THE SYMBIOTES FOUND IN ANIMAL EMBRYOS

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PART I: AN INTRODUCTION.

It would be difficult indeed to find in all of parasitology a subject delimited by such narrow margins as the one to be treated in this thesis. The symbiote is limited to a locale quite out of the way and, in most cases, minute. Special conditions are necessary in order for the symbiote to enter the egg or embryo. Eggs and embryos are well protected from marauding predators and symbiotes. Morphologically, the reproductive system's organs are so constructed that reaching their innermost lumina is a difficult feat. The reproductive system is quite isolated from the external environment even though it has a connection to it, the genital pore. Within the canal there are usually cilia which beat towards the opening, secretions deleterious to prospective symbiotes, and other defenses which serve to protect the reproductive organs from marauders. Endosymbiotes of the adult host find it difficult to reach the reproductive organs unless they are tissue-cutting forms; even the blood inhabiting forms cannot invade unless the gonadic vessels rupture. The egg and the developing embryo are protected in most oviparous animals by a tough shell. It is true that many aquatic vertebrates lay naked unfertilized eggs which are open to invasion but these eggs apparently do not have a proportionately larger number of symbiotic inhabitants.

In this thesis there will be no attempt to enumerate all the symbiotes found in all embryos or eggs since such an undertaking would require years of library research. Parasitology

is a young discipline although certain common parasites have been known for centuries (e.g. Dracunculus medinensis, the fiery serpent of the Old Testament). New symbiotes and new relationships are being discovered daily. Information pertaining to host-symbiote relationships of a purely scientific nature and having no economic value are hidden away in obscure journals while transovarial transmission of symbiotes of economically important animals is over emphasized in parasitological literature.

Before going on it would be well to define some of the terms used in this thesis. Following the recommendation of the Committee on Terminology of the American Society of Parasitologists (Journal of Parasitology, 1937, 23:326-329.) the word "symbiosis" will be used as de Bary (1879) originally used it, that is, as a general term referring simply to the living together of dissimilar organisms, not excluding parasitism, mutualism or commensalism. The term symbiote was also found to be preferable and more correct than the word "symbiont". Cleveland (1926) had anticipated this when he wrote that in true parasitism either the host of the parasite is injured. He also wrote: "It would be much better, however, to use the term symbiosis, which means living together, and under it include commensalism, mutualism and parasitism.... This partnership (symbiosis) may be between animal and plant, plant and plant, and animal and animal." In this thesis the emphasis will be upon relationships of animals with animals.

The Committee on Terminology also stated that:

> "Lack of unanimity among helminthologists may be interpreted as a tendency toward abandoning the term infestation.... We believe that infest and infestation ought to revert to their original use in connection with external, and in most cases visible agents....We believe the terms infect and infection properly applicable wherever the parasite invades and establishes itself within the body of the host, including, in this sense, the gastro-intestinal tract."

Resistance, according to Taliaferro (1926), may be used in the broad sense to denote various mechanisms, either active or passive, of the host to counteract the activities of the invading parasite.

The term "embryo" will be limited in this thesis to any stage in the development of the fertilized egg incapable of obtaining food from external sources. This necessarily excludes the nymphs and larvae of the arthropods and some immature stages of the other invertebrates. Among the vertebrates with external embryonic development it includes all stages up to the formation of the mouth by rupture of the stomodaeum. This definition necessarily includes all the intrauterine stages of the higher vertebrates.

THE PROBLEM:

The subject matter has been broken up into three sections: the Protozoa, the helminths, and the arthropods. These three groups which include most of the described species of animals also contain most of the symbiotic forms. The few symbiotes that do occur in the other animal phyla will not be considered here. For example, a hydrozoan coelenterate, Polypodium, is symbiotic for part of its lifetime in the ovary of the sturgeon. (Parker and Haswell, 1940).

It is intended to include in the discussion that follows the symbiotes found in the gonads because of the possibility that these may wander into the developing ova or be transmitted from male to female along with the male gametes and so get to the developing ova. Included also is a lengthy survey of some of the plant and plant-like symbiotes of arthropods. Transovarial transmission of these symbiotes has been studied in great detail and provides many fine examples of special embryonic structures so designed as to provide adequately for the new guests. Included also is a section of the entomophagous egg parasites. This section also will be incomplete due to the great numbers and diversities of insects that participate in this hateful occupation.

Part V of the thesis is a summary of the author's attempts to experimentally infect chick embryos with the common fowl nematode, Heterakis gallinae (Gmelin 1790) Freeborn 1923.

PART II. THE PROTOZOA:

Calkins (1926) states that the distribution of the symbiotic forms belonging to all groups of the Protozoa obviously follows the distribution of their hosts. Where animals are segregated the opportunities for symbiosis are enhanced. Thus the blood-dwelling parasites are more common in the tropics than elsewhere, the biological conditions favorable to the intermediate transmitting hosts being largely responsible for their numbers and variety. It seems the Protozoa (not unlike most lower animals) prefer a fluid or semifluid environment. They live not only in the exposed waters of the earth's surface but also as symbiotes in living protoplasm or its products. No type of animal life is free from the possibility of association with Protozoa as symbiotes but host-specificity seems to be the rule. While most of these animals are free-living, the Class Sporozoa is exclusively symbiotic. The sporozoans are exclusively coelozoic, histozoic or cytozoic symbiotes.

A great many of the Protozoa treated in the following pages are symbiotes of arthropods. Some are pathogenic and long have been the concern of parasitologists and medical researchers. The two most common methods of transmission of the protozoan diseases of insects are by way of the mouth, and from the female through the egg to the next generation. Transmission through the egg occurs with many species (see Table 1) and in many of these it is the principal method of transfer. In other species such as _Perezia pyraustae_, transmission can take place

very readily through the contamination of the host's food as well as through the eggs. The protozoans found in the Acarina are not host-specific like those of the Hexapoda since most are also symbiotes of other animals.

There seems to be some uniformity of size among the symbiotic protozoa. The symbiotic forms especially the cytozoic symbiotes, are often extremely small. Plasmodium, Leishmania and microsporidian spores may be mentioned as examples of the smallest protozoan forms. Like certain symbiotes higher phylogenetically a number of protozoans have developed special organellae for attachment. Many genera of cephaline gregarines are provided with an epimerite of different structure by which the organisms are able to attach themselves to the gut epithelium of the host. Other developments for anchorage such as chitinous structures (Astomata), suckers (Giardia), and polar filaments (Myxoxporidia) are also found among the symbiotic Protozoa but need not concern us here. The contractile vacuole which serves for excretion is absent in the Sporozoa which are all symbiotic and the Sarcodina and Mastigophora which live in the body of other animals. In the cytosome of many symbiotic flagellates, there is frequently present a conspicuous structure known as the parabasal apparatus. This structure varies greatly among different genera and species in appearance, structure and position within the body.

The Protozoa which live within the body of another organism are able to nourish themselves by absorbing the

digested or decomposed substances of the host and could be considered as saprozoic. Coelozoic Protozoa like the Protociliata, astomous ciliates and the Trypanosomatidae belong to this group. In the case of certain cytozoic forms such as the Cnidosporidia, the host cytoplasm is apparently liquefied or hydrolyzed by enzymes (Kudo 1939) before being absorbed by the symbiote. The symbiotic Protozoa which actually feed on host tissues, such as Entamoeba histolytica Schaudinn, Balantidium coli Stein, etc., or endocommensals, employ the holozoic form of nutrition.

Respiration among those Protozoa which happen to be symbiotes of developing eggs and embryos necessarily must depend upon the respiratory processes of the host animal. Endozoic Protozoa live in an isotonic medium. Hence there is no excess of water entering the body and therefore there is no need for contractile vacuoles. (Just why all euciliates possess the contractile vacuole regardless of habitat has never been explained.) In the case of symbiotic Protozoa, possibly the reaction to chemical stimuli results in their distribution in different organs and tissues within the host body.

Subclass Phytomastigina

In this subclass of the Class Mastigophora the only group that need concern us is the Order Dinoflagellata Bütschli. Members of this order seem to limit their symbiotic relationships to the higher forms of Invertebrata. Most of the bits of information concerning this group presented here were gleaned from a monograph written by Chatton (1920). Some

erratic members of this group, however, are found in various structure of radiolarians and oligotrichs. The symbiotes are found, for the most part in the eggs of pelagic copepods in the Bay of Naples and at Banyuls-sur-Mer. The results of these geographically-limited studies hint that many more such relationships between Copepoda and Dinoflagellata exist in the plankton of the world's seas.

The Genus Chytriodinium Chatton will serve as an example of a Dinoflagellate egg-symbiote. The flagellates reside in the eggs of planktonic Copepoda. The young individuals grow at the expense of the host egg and when fully formed, the body divides into many parts, each producing four swarmers. These swarmers then invade new eggs. The penetration of the egg by a swarmer is not exactly known as is shown by the following quotation taken from Chatton (1920):

> "L'hypothèse de la pénétration du parasite dans l'oeuf n'est pas sans laisser l'auteur dans l'embarras, car il n'a pu y constater 'de visu' la présence du parasite. Il suppose qu'il y pénètre et y demeure à l'état amoeboide, plus ou moins confondu avec le cytoplasme de la cellule hôte....Quoi qu'il en soit, on voit que les oeufs des Copepodes pelagiques et les Protistes peuvent être attaqués de deux manières très différents par des Péridiniens parasites. Tandis que les Chytriodinium et Paulsenella se developpent sur la cellule à la manière des Chytridinées du genre Chytridium, Trypanodinium et Duboscquella croissent et sporulent â son interieur à la manière des Olpidium."

A summary of some other Dinoflagellate symbiotes of eggs can be found in Table I.

Subclass Zoomastigina

The order Rhizomastigina Butschli contains one symbiote of

note, *Histomonas meleagridis* (Smith), the cause of "blackhead" disease of fowl (Tyzzer, 1919). It attacks the intestinal mucosa as well as liver tissues. Graybill and Smith (1920) showed that the embryonated eggs of *Heterakis gallinae* (Gmelin 1790) Freeborn 1923 can transmit blackhead disease of turkeys. The protozoan has not been found in the infected stages of the nematode. It is not known to form cysts but it can be transmitted not only with the feces and diseased tissue but also by the embryonated eggs of the cecal nematode. Niimi (1937) claims to have seen *Histomonas* in the eggs of *Heterakis*. *papillosa* Bloch. Miss Bishop (1938) made careful experiments infecting young chickens with embryonated eggs of *Heterakis*. The chicks subsequently developed typical "blackhead" infections. More will be said concerning this symbiote in Part V, the section devoted to the author's work on the experimental infection of chick embryos with the *Heterakis gallinae* from a hen suspected of being a carrier of "blackhead" infection.

The second order of the Zoomastigina, the Protomonadina, has two representatives symbiotic in the developing eggs and embryos of arthropods. *Crithidia hyalommae* O'Farrell is found in the body cavity of the tick *Hyalomma aegyptium* (Linn.) in Egypt. The flagellate through its invasion of the ova (in leishmania form) is said to be capable of infecting the offspring while it is still in the body of the parent tick (Kudo, 1939). *Leptomonas jaculum* is found in *Nepa cinerea* Ferr., a water scorpion, and has been observed penetrating the eggs of

its host (Calkins 1926).

Much work on the trypanosomes has been accomplished by the experimental infection of developing chick embryos with these flagellates. Unlike some of the other monomorphic trypanosomes *Trypanosoma cruzi* (Chagas) does not multiply in the chorioallantoic fluid of developing chick embryos, though it may survive for a number of days in that environment. It will, however, grow in chick embryo tissue cultures. Prenatal infection by this symbiote has been reported in dogs, and one human case reported by Mazza (1939-42) was apparently acquired with the mother's milk.

The African polymorphic trypanosomes such as *T. gambiense* Dutton, *T. brucei* Plimmer et Bradford, and *T. rhodisiense*, are not easily cultured in the usual artificial media but thrive when inoculated into developing chick embryos, a rich, living culture medium.

At least one trypanosome, *T. equiperdum* Doflein, has become completely independent of its ancestral invertebrate hosts and is transmitted directly from horse to horse during copulation. It thrives in the edematous fluids of the sex organs and the skin and causes the condition known as dourine. *T. hippicum* in Panama is said to be transmitted both by vampire bats and venereally as is *T. equiperdum* (Chandler, 1944).

Many species of the genus *Cryptobia* Leidy are symbiotic in the reproductive organs of certain molluscs and other invertebrates.

The next order, the Polymastigina, has one genus which interests both the veterinarian and gynecologist alike, namely, the genus Trichomonas Donne. Trichomonads vary in their pathogenicity from the harmless coprozoic form, T. fecalis Cleveland, to highly pathogenic species like T. foetus (Riedmüller), which is an important cause of abortion in cattle, and T. gallinae which causes a considerable mortality in pigeons and other birds. Fortunately, the pathogenicity of the species found in man is relatively low.

Trichomonas foetus (Riedmüller) has been found to be a common and injurious symbiote in the genital tract of cattle; it can be experimentally established in sheep also. It is a venereal disease, transmitted from infected bulls to heifers and vice versa. It attacks the mucous membrane of the heifer's vagina and invades the uterus, causing abortions, stillbirths, delayed conceptions and other damage. It also inhabits the prepuce and other parts of the genital tracts of bulls. After a number of months the animals overcome the disease and are immune to further infection. Rees (1938) and Kerr (1943) have made extensive studies of this disease.

Trichomonas vaginalis Donne, an inhabitant of the human genital tract chiefly that of the female, has received much attention in recent years because of its suspected pathogenicity. Experimentally it has been maintained in developing chick embryos by McNutt and Trussel (1941). Relatively large inocula into the allantoic sac were required to maintain the organism

for 24 subcultures. No pathogenic effect was noticed. By comparison, T. foetus (Riedmuller) was much more readily carried through successive embryos with smaller inocula. T. vaginalis has also been found in ovarian cysts. Marconi (1935) inoculated guinea pigs, rats and mice with T. vaginalis and observed that a few pregnant animals aborted. A few non-pregnant animals subsequently failed to conceive. Yet he never could demonstrate the survival or multiplication of the protozoan. Hees (1938) claimed that he had isolated T. ardin-delteil (a pentatrichomonad) from human and bovine fetal blood and T. vaginalis from human fetuses. Rodecurt (1937) also found T. vaginalis in aborted fetuses. These findings have been questioned and denied by many authors. Unpublished data collected by Trussel (Trussel, 1947) support the belief that trichomonads are not significantly involved in human abortion. There is no question about the pathogenicity of T. foetus. Morgan recently (1943) counted these trichomonads in the amniotic and allantoic fluids of an aborted four months bovine fetus. He found 2,080,000 and 1,120,000 organisms per cc. respectively.

An interesting case of hyperparasitism was discovered by Hunninen and Wichterman (1938). They found that the reproductive systems and especially the eggs of intestinal trematodes (Deropristis inflata Molin, 1858) from the marine eel Anguilla chrysypa Rafinesque 1896 were found "parasitized" with Hexamita Dujardin. Heavily "parasitized" eggs of the trematode did not

develop into miracidia.

According to Kirby (1937) the order Hypermastigina Grassi is made up of those species that are inhabitants of the alimentary canal of the termite or other insects. Every species of four of the five families of Isoptera (Mastotermitidae, Hodotermitidae, Kalotermitidae, and Rhinotermitidae) contains flagellates in the hindgut, and these are present at all stages of the life history except the eggs, the youngest instars, the specialized reproductives of certain species, and in each member of the colony preceding of immediately following a molt. Due to their caprophagous habits the termite larvae pick up their normal intestinal fauna a day after hatching. The colonial habits of these insects has eased the problems of transmission of the symbiotes and transovarial transmission has not had to be resorted to.

The Class Sarcodina has no known representatives which live symbiotically in eggs or developing embryos of animals.

The Subclass Telosporidia of the Class Sporozoa contains many species that are transmitted hereditarily. The Class Sporozoa are without exception symbiotic and bear spores in their development. Their nutrition is either saprozoic or parasitic. The first order, the Gregarinida, are chiefly coelozoic symbiotes of invertebrates, especially arthropods and annelids. The family Monocystidae is of especial interest. The species of this family have, for the most part, taken up an abode in a strange location, namely, the seminal vesicles of oligochaete

annelids. A monograph by Hesse (1909) discusses this strange group. It seems the symbiotes do not invade the gametes but content themselves by floating in the nutritious seminal fluids and at times devouring sperm cells (Monocystis herculea, Bosanquet). Hesse writes:

> "Monocystis lumbrici accomplit ainsi tout son cycle evolutif sans pénétrer dans les cellules sexuelles de son hôte; elle se nourrit simplement aux depens du fluide seminal par osmose."

Hesse noted that more of the gregarines are found during the mating season of the host worms and the following is his explanation of the phenomenon:

> "...ce développement des parasites est commandé par le développement même des cellules qui serve a les nourir."

Many of the gregarines are parasitic in the larva of insects and therefore are of economic importance. A cursory tabulation of some of the gregarines found in the seminal vesicles of annelids will be found in Table I. This list has been appended to the list of known protozoan symbiotes of the eggs of animals because these Protozoa are potential invaders of the gametes. Since the exact method of transmission of these symbiotes from one hermaphroditic worm to another is unknown there is some justification in appending the list. Gonospora minchini Goodrich et Pixell-Goodrich is the only gregarine known to be symbiotic in the ova of an annelid. It lives in the coelom of the polychaete, Arenicola ecaudata. The young trophozoites live in the host eggs when they are fully grown. Many other gregarines live in the gut tissue of larval insects but are

probably infections acquired *per os*.

The order Coccidia (Subclass Telosporidia) has a wide zoological distribution. The majority are symbiotes of the epithelium of the digestive tract and its associated glands. *Caryotropha mesnili* Siedlecki lives in the coelom among the floating bundles of spermatogonia of the polychaete, *Polymnia nebulosa*. The cysts of this protozoan are extruded with the reproductive cells of the host worm. Another coccidian of interest is *Karyolyssus lacertarum* (Danilewsky) which is a symbiote of the lizard *Lacerta muralis*. The sexual reproduction of this protozoan takes place in the female mite, *Liponyssus saurarum* Oudemans, in which the sporokinetes enter the ova and mature. During the tissue differentiation of the mite embryo the sporocysts occupy cells which become the gut epithelium. After the mites first blood meal the spores are cast out of the body, and when ingested by a lizard, this animal becomes infected (Kudo, 1939).

The order Haemosporidia Danilewski contains one genus of relevance here. Members of this group are minute intracorpuscular symbiotes of vertebrates. *Babesia bigemina* (Smith et Kilbourne), the causative agent of Texas fever, is transmitted by the tick *Boöphilus* (*Margaropus*) *annulatus* Say. This fact was discovered by Smith and Kilbourne in 1889 and is of great historic as well as practical importance since it was the first demonstration that an arthropod was capable of transmitting a protozoan. Likewise startling was the finding that

the symbiote was transmitted to the next generation through the egg. This method of transmission was evident by the fact that the tick remains on the one host through all the stages of development and only drops off as an adult to lay its eggs. Recent work has been done on this symbiote by Dennis (1932). Crawley (1915) reported finding numerous club-shaped bodies which he described as "gregarinoids" in the ova of Margaropus annulatus, which were presumably infected with B. bigemina. Dennis traced Babesia through the egg and into the larva of the tick by isolating infected, gravid female ticks in large test tubes which contained moist sand, and allowing them to oviposit for three or four days. He removed the eggs daily and incubated them. Smears, squash preparations and sections of developing eggs were made.

The anatomy of the tick facilitates this transovarial transmission. In the engorged female there is a space of about five micra between the lumina of the gut and the oviducts. The female reproductive system is wound in and out among the diverticula of the gut so that some part of it is in close contact with or very close to some branch of the blind gut at almost all points. The fact that the symbiotes enter the eggs is apparently not a matter of tropism or preference; there is essentially no other place to go. Sporogony of B. bigemina occurs in the tick. In his embryological studies Dennis found that certain stages (sporokinetes) migrate throughout the tissues of the developing tick, and since much of the embryonic

cell mass contributed to the formation of the salivary glands, it is almost inevitable that some of the symbiotes should come to lie in these structures, whence they may be transferred to a new host during feeding.

Another Babesia, *B. canis* (Piana et Galli-Valerio), passes through the eggs of ticks (*Rhipicephalus sanguineus* (Latr.) in the United States and India, *Dermacentor reticulatus* (Fabr.) in Europe and *Haemaphysalis leachi* (Audouin) in South Africa) to the next generation.

The Anaplasmata is a group of organisms of doubtful nature transmitted by arthropods. For convenience they have been placed in with the Protozoa. They are small coccus-like bodies or granules on the margins of the erythrocytes of cattle. Their generic name refers to the apparent lack of cytoplasm about the symbiote. Two species have been described, *Anaplasma marginale* Nieles, 1910 and *A. centrale* Theiler, 1910. Practically nothing is known concerning the life cycle of *A. marginale* in its arthropod hosts. It is well known, however, that in the ticks, *Boöphilus annulatus* (Say), *Dermacentor occidentalis* Neum. and *Ixodes ricinus* (Linn.), the agent passes through the egg to the next generation.

The next subclass of the Sporozoa, the Cnidosporidia, is an especially fruitful one for a person seeking to find symbiotes of embryos. Members of this group are exclusively parasites of the lower vertebrates and invertebrates. The symbiotes of the order Myxosporidia are limited to the lower

vertebrates. One species, *Agarella gracilis* Dunkerly, takes up its abode in the testis of the South American lungfish *Lepidosiren paradoxa*.

The cosmopolitan order Microsporidia contains species which are typically intracellular symbiotes of arthropods and fishes. The effect of these protozoans is hypertrophy of the host cells. The most famous member of this order is *Nosema bombycis* Nägeli, which is found in all tissues of the embryo, larva, pupa and adult of the silkworm, *Bombyx mori* (Linn.). It is the causative organism of pébrine disease of the host caterpillar. When the maternal infection is heavy, the embryo cannot complete its development and dies inside the egg-chorion. The lack of normal nutrition on the part of the host is the main cause of its demise. Other insects also suffer from related microsporidioses. The honeybee, some lepidopterous pests, and mosquitoes are known to harbor symbiotes of this type while yet very young. For example, *Plistophora stegomyiae* Chatton, 1911 is a symbiote of both larvae and adults of *Aëdes aegypti* (Linn.) The most frequent method of new infection seems to be through the eggs, although infection through the mouth also occurs. Kudo (1924) states that there are two modes of microsporidian infection, *per os* and through the eggs. No intermediate host animals had up to that date (1924) been found for the Microsporidia. The infection of a new host animal usually takes place when the latter ingests spores of a specific microsporidian capable of germinating in its gut. Autoinfection also occurs.

Since Pasteur (1870) established germinative infection in *Nosema bombycis*, Nöller (1912) found that it invaded not only the host digestive tract, but also ovary and ovarian eggs of *Ctenocephalus canis* (Curtis), and suggested that the symbiote was carried from generation to generation through germinative infection. Flu (1911) in *Musca domestica* Linn. and Chatton and Krempf (1911) in *Drosophila confusa* Staeger and *D. plurilineata* noted that *Octosporea muscae-domesticae* Flu invaded the yolk of the egg, and suggested there might be germinative infection. Pérez (1906) reported heavy infection of the ova of a host crab by *Thelohania maenadis* Pérez 1904, which showed absolute normality:"...Les ovules étaient chargés de vitellus, et selon toute vraisemblance auraient été prochainement pondus." With *Plistophora stegomyiae* Chatton, 1911, Marchoux and Simond (1906) noticed that ova infection was quite frequently seen in host mosquitoes. According to Christophers (1901) and Nicholson (1921) the eggs of *Anopheles maculipennis* Meig. are frequently found loaded with a sporozoan. There seems to be little doubt in such a case the germinative infection will take place if the infection is moderate so that it does not affect the development of the host embryo. There are many other examples of symbiotes of embryonic animals in the Microsporidia. These can be found in Table I. Some of the forms mentioned in Table I that are symbiotes within the gonads (e.g. *Thelohania giardi* Henneguy 1892 and *T. octospora* Henneguy, 1892) cause a phenomenon known as parasitic castration of their crustacean hosts.

The author has read of only two ova symbiotes in the entire Subphylum Ciliophora. Both are found in the subclass Euciliata, the first in the order Holotricha the seond in the order Peritricha. According to Kudo (1939) _Espejoia_ _mucicola_ (Penard) is found in the gelatinous envelope of the eggs of certain insects and molluscs. The peritrichous genus _Lichnophora_ Claparede is, according to Calkins (1926), an ova ectosymbiote of _Crepidula_ sp..

PART III: HELMINIHS SYMBIOTIC IN ANIMAL EMBRYOS

Worm infections differ radically from bacterial or protozoan infections in that the worms do not multiply in the body of the host, and so the infections are quantitative in nature; the severity of the infection, therefore, is not controlled almost entirely by the resistance of the host, which varies from time to time, but it depends very largely on the actual number of worms acquired. Another difference is that only those forms in which the transfer from the intermediate to the definite host is direct (that is, the intermediate host is the food of the final host) and in which the definite host or its excreta immediately reach the larval host, are independent of a continuously moist environment. As Faust (1947) aptly puts it: "Moisture is the sine qua non for the majority of helminthic infections."

The principal ways in which helminths harm their hosts are by mechanical damage, by devouring tissues, and by toxic effects. The role of the worms as vectors for viruses and other infectious agents is still almost unstudied.

Hyperparasitism was seen in some of the protozoa discussed but the worms are more susceptible to such symbiotic states. The nematodes are well known to have their symbiotes but the trematodes according to Dawes (1946) seem to be less liable to the condition termed hyperparasitism.

For the sake of convenience the discussion will be divided into two parts, namely, the Platyhelminths or flat worms and the Nemathelminthes or roundworms which also includes

the acanthocephaline worms. The annelids include only one characteristically symbiotic group, the Hirudinea, but this group will not be treated in this thesis because the leeches are occasional symbiotes. They feed on blood of various animals to which they attach themselves and drop off after having engorged. Their manner of obtaining nutriment and the nutriment itself precludes any possibility that they may be symbiotes of eggs or embryos. The horsehair worms, the Nematomorpha, whose larvae attack and mature in the tissues of insects have also been excluded because in the author's reading no evidence of horsehair worm as a symbiote of either egg or of embryo was found. The class Turbellaria of the Platyhelminthes is composed for the most part of free-living forms and for this reason has not been investigated.

The Trematoda

Before starting on this extensive group it must be acknowledged that most of the material on the class was obtained from the comprehensive monograph on the Trematoda by Dawes (1946).

The Trematoda is broken up into three subclasses, the Monogenea V. Beneden 1858, Aspidogastrea Faust and Tang (1938), and the Digenea V. Beneden 1858. The group that will most concern us is the Digenea. But first a short glance at the other two subclasses.

The Monogenea v. Beneden 1858 are all ectosymbiotes or in the excretory bladder or respiratory passages of the host. In the Monogenea, a single egg represents one potential adult, but in the Digenea the egg shelters not one, but a host of potential individuals. Members of the Gyrodactylidae may differ from other Monogenea in being viviparous. The egg of *Gyrodactylus elegans* Nordman, 1832 according to Dawes does not become encapsulated, but develops into an embryo within the body of the parent. Before the embryo is born, even before it has taken on the characters of the parent, it has given rise to a smaller embryo within itself, and this in turn to a still smaller embryo which contains a fourth and smallest embryo. This situation would remind one of a Chinese puzzle box and if the author wished to stretch the meaning of embryo and symbiote he would have another example of a symbiote of an embryo. There is one

veritable example of an egg symbiote among the monogenetic trematodes and that is Udonella caligorum Johnston 1835 which lives mainly on the egg sacs of female copepods of Caligus spp.

The Aspidogastrea are symbiotes on or in the soft parts of molluscs, or in the intestinal tract of cold-blooded vertebrates. The present author knows of no embryo-symbiotes among this group.

The digenetic trematodes not only have an alternation of generations (metagenesis) but also an alternation of hosts. The host of the generation producing fertilized ova (the marital generation) is usually a vertebrate; the intermediate host is always a mollusc. The trematodes which live as intestinal, bile passage, or lung symbiotes get in as encysted metacercariae which are ingested in the host's food or drink.

Since the intermediate hosts of the Digenea are molluscs it would be well to survey the asexual cycles of these animals to see whether at any time some may take up residence in the ova or developing embryos of their hosts. Cercaria himasthela secunda Sinitsin, 1905 and Cercaria lophocerca Rees attack the gonad of Littorina litorea L. (a snail). The gonad is eaten by the larvae and disappears - an example of the phenomenon known as "parasitic castration". The gut of the larva is frequently filled with eggs and yolk. According to Vickers (1940) Cercaria macrocerca Filippi, 1854 is found on Sphaerium corneum (Lamarck) between the gill lamellae together with the developing embryos of the snail. Timon-David (1938) reports that

Metacercaria capricosa (Cuenot 1893) occurs on the gonads of the Ophiuroids *Ophiothrix fragilis* and *Ophiura albidae* at Roscoff. *Cercaria echinatoides* (Filippi 1854) is symbiotic on the gonads and heart of *Paludina vivipara* (Lühe 1909). The larvae of *Ptychogonimus megastoma* (Rudolphi 1819) invade the tubules of the testes, the vas deferens, and the coelom (near the ovary) of the Crustacean, *Brachyura*. Herber (1939) found that the tadpoles of *Rana clamitans* can be infected with the cercariae of *Diplodiscus temperatus* Stafford when ten days old or as soon as the mouth becomes open. Inclusion of the previous example may not be justified because a ten day old tadpole may or may not be considered still an embryo.

The general cloacal region of the Aves seems to be especially plagued by the presence there of numerous symbiotic digenetic trematodes. The oviducts and bursa Fabricii are subject to invasion by a great number of these flukes. The maritae of the family Plagiorchidae Lühe 1901 found in the intestine, buccal cavity, lungs and oviducts of amphibians, reptiles, birds and mammals. The oviduct flukes of birds are of economic as well as biological importance. The genus *Prosthogonimus* Lühe, 1899, belonging to family Plagiorchidae, Lühe 1901, inhabit the oviduct and bursa Fabricii of birds. Several species, including *P. macrorchis* Macy, 1934, in the north-central United States, are important parasites of poultry, causing a marked falling off in egg production, and sometimes fatal disease. The cercariae of these flukes, after

developing in snails of the genus *Amnicola* Gould and Haldeman, encyst in dragonfly nymphs. The birds become infected by eating either nymphs or adults of dragonflies. Szidat (1927) reported that *Prosthogonimus intercalandus* Hieronymi and Szidat, 1921 causes a fatal inflammation of the oviduct of fowl, or, at least, the production of defective eggs. *Plagiorchis arcuatus* Strom is found in the oviducts of fowl in Russia and Germany. *Prosthogonimus ovatus* (Rudolphi 1803) is found in the bursa Fabricii and sometimes the oviduct and eggs of fowl, geese, and various wild birds (Theobald, 1920). It is a widespread species and is pathogenic, causing inflammation of the oviduct and various abnormalities of the egg (in which it is sometimes included) and egg production. It often induces a fatal peritonitis. *P. cuneatus* (Rudolphi, 1809) Braun, 1901 is similar to *P. ovatus* but is also symbiotic in the swan. *P. pellucidus* Linstos is a symbiote of the bursa Fabricii and oviducts of fowl and curlew.

Perhaps the most publicized of all flukes is *Fasciola hepatica* L., the sheep liver fluke, which occurs in sheep, goats, cattle, other ruminants, pig, hare, rabbit, beaver, copyu, elephant, horse, dog, cat, kangaroo and man and perhaps many others. Some of the young flukes that enter the bloodstream may become trapped in unusual sites and may, according to Mönnig (1947), even infect the embryo in pregnant animals. The findings of prenatal fluke infection among humans have led certain helminthologists, among them Braun (1925) and Bügge (1928) to conclude that the worms enter the portal system and

from there are distributed throughout the body. This seems to be the most reasonable explanation for the findings of the flukes in these abnormal foci.

By the use of secretions of the penetration glands and the activity of the anterior organ of penetration, the cercaria of the family Schistosomatidae Looss, 1899 pass swiftly through the skin as Looss first demonstrated on himself, enter the lymphatics or blood vessels and ultimately congregate in the venules of the hepatic portal or other appropriate system. In pregnancy they may pass from the maternal to the foetal blood vessels in the placenta, so that the newly born animal is already in the first stages of schistosomiasis. Table III contains a list of the embryosymbiotes of this family and their hosts. Human schistosomiasis due to *Schistosoma japonicum* Katsurada, 1904 may be contracted as a prenatal infection. In 1916 Narabayashi reported eggs of this infection from the stools of three newly-born babies, whose mothers worked in the rice fields in endemic areas in Japan. More recently Hovard (1933) reported infection in a two week old infant of an Asiatic family traveling in British Guiana.

A few trematodes take part in phenomena known as hyperparasitic relationships. *Udonella caligorum* thrives on the ovisacs and posterior region of species of *Caligus* (Crustacea), itself a symbiote of the cod and other gadoid fishes. Hunninen and Wichterman (1938) found a flagellate of the genus *Hexamita* to be parasitic in the eggs and uterus of the trematode

Deropristis inflata, which in turn is a symbiote of the marine eel, Anguilla chrysypa Rafinesque, 1896 (see p.12). The hyperparasites occurred also in the oviduct, receptaculum seminis, vitellaria and testes. In several instances counts revealed the fact that all the eggs were infected, and it was found that heavily infected eggs (twenty or more flagellates per egg) did not develop miracidia. Brumpt reported on infection of Fasciola hepatica and Fasciolopsis buski, which may be destroyed by a fungus, Chytridiacea, and Butler and Humphries (1932) found that the eggs of the former together with gelated white of egg make an ideal culture medium for the fungus Catenaria anguillulae. Before passing on to the Cestoda reference must be made to Table II. This table is a listing of the worm embryo-symbiote relationships gleaned from the rècent literature. Included also is a listing of the symbiotes of the bursae Fabricii of birds since it would not be too difficult for a symbiote to find its way into the oviduct of the avian host and become a symbiote of the developing egg. The inclusion of this list then is perhaps justified.

The Cestoda

This group need not occupy us long for there are no clear-cut examples of embryo symbiotes in the class Cestoda. Monnig (1947) writes that cases of infection by Taenia saginata Goeze 1782 in calves suspected to have occurred before birth have been reported but he fails to give references. Faust (1939) also is noncommittal. He says that on consuming insufficiently cooked flesh and possibly the roe (caviar) of infected fish, man is exposed to Diphyllobothrium latum (L. 1735) infection (sparganum infection). He also says that Sparganum proliferum (Ijima 1905) is found in all tissues in the human except the osseous tissues. Chandler (1944) states that Echinococcus granulosus (Batsch 1786) prefers the liver and lungs to develop in. Smaller numbers reach the kidneys, spleen, intestinal walls, peritoneal lining, genital organs, heart, brain and various muscles. Houttuynia struthionis (Parona 1885) is highly pathogenic for ostrich chicks but the author was unable to find recorded instances of prenatal infection.

The Nematoda

The nematodes are symbiotes scattered throughout the world in almost every kind of metazoan animal. Their distribution necessarily follows that of their hosts. Cobb has calculated that 80,000 species are symbiotic in vertebrates and Baylis estimates that 300 genera are symbiotic. As in most biological statistics the correct answer probably lies between the two estimates. To review the habitats of all symbiotic species in order to extract the names of those dwelling in embryonic tissue would be an almost insurmountable task. The examples cited below are all commonly known worms and material pertaining to them makes up the bulk of the material on the nematodes.

Unlike many tissue-penetrating larvae, *Trichinella spiralis* (Owen) embryos do not usually pass through the placenta and cause prenatal infections although several authors unsuccessfully attempted to produce prenatal infection with this symbiote. Roth (1936) found a small percentage of foetal guinea pigs infected after heavily infecting the pregnant mothers.

Toxocara canis (Werner 1782) is a symbiote of the alimentary tract of dogs and foxes. It is highly pathogenic for the pups and intra-uterine infection of the pups by the larvae of *T. canis* which pass through the lung capillaries of the mother and thus get into her systemic circulation is not rare. Such larvae remain in the liver of the foetus until the

latter is born and begins to breathe, when they continue their migration through the lungs. In the case of the fox pups the most dangerous period for this infection is during the first few weeks of life. Sometimes the ascarids migrate into the peritoneal cavity of the pup and thus cause extreme potbelliedness.

Pearl et al. (1921) wrote that occasionally _Ascaris inflexis_ Rudolphi 1809 (synonym _Ascaridia galli_ Schrank 1788) or "other" roundworms may pass from the cloaca into the oviduct. In this way they may be incorporated in the albumen of the egg, as it is formed in the oviduct.

Ascaris lumbricoides L. occurs in the small intestine of man, certain large apes, and the pig. Its intra-host life starts with the ingestion of the eggs. These hatch in the intestine and the larvae burrow into the wall of the gut. They sometimes pass through into the peritoneal cavity and thence to the liver, but usually reach this organ by way of the blood stream. From the liver they are carried by the hepatic vein to the heart and then to the lungs where they are arrested because of their size in the capillaries although some may pass through the lungs and into the systemic circulation and thus reach other organs like the spleen and kidney and even the foetus in a pregnant animal. Lapage (1937) reports that they have been found in the pleural cavity, vagina, bladder and uterus. In some of these instances larvae probably penetrated into and developed in the coelom, but this view would require the assumption that _Ascaris_ can

develop in a sterile environment.

The effects of hookworm infection are well known but they are particularly severe during pregnancy when the demand for iron by the developing foetus puts an extra drain on the mother. Wickramasuriya (1935) in his study of hookworm-infected women in Colombo, India reckons this disease to be a more serious complication of pregnancy and normal birth than syphylis, eclampsia and puerperal sepsis. The worm then does not attack the embryo directly but by its drainage of the iron supply of the embryo causes destruction. Thus the worm in this sense is an indirect symbiote of the embryo.

Ancylostomiasis and uncinariasis (caused by Uncinaria stenoscephala (Railliet, 1884) occur chiefly in the summer and especially in animals that are confined on a relatively small area of moist ground, like dogs in kennels and foxes in runs. Both conditions are seen in animals of all ages, but usually not, like ascariasis, in very young pups. Prenatal infection, however, may cause a sudden onset of severe anemia, coma and death about three weeks after birth.

Another nematode that sometimes gets lost in its wandering about the host body is Dictyocaulus filaria (Rudolphi). This worm occurs in the bronchi of sheep, goats, and wild ruminants. The infection of the host occurs per os. The larvae penetrate into the wall of the alimentary canal and pass by way of the lymph vessels to the mesenteric lymph glands. The worms now travel via the lymph and bloodvessels to the lungs

where they are stopped in the capillaries and break through into the air passages. Some larvae may pass through the pulmonary capillaries into the systemic circulation and set up a prenatal infection in an embryo (Mönnig 1947).

An interesting variation is the experimental determination of the presence of Habronema megastroma (Rudolphi, 1819) in the stomach of equines. Descazeaux and Morel (1933) recommend incubating the feces of suspected animals with washed eggs of the house fly and later finding the larvae in the flies. It is obvious that the embryonic flies acquire the symbiote during their development in an environment containing viable eggs of the helminth. It must be added, however, that this xenodiagnosis is good only in the warm season.

Enterobius vermicularis (L.) has never been recorded as a parasite of a human fetus but there are several cases on record in which the gravid female worms have migrated through the vagina and Fallopian tube of female patients, where they have become encysted. Wu (1935) reports that in the tubules they may produce symptoms simulating salpingitis of gonococcus or M. tuberculosis origin.

The Nematomorpha (or Gordiacea) are interesting biologically in that the immature larval stage is symbiotic in various insects, while the adults are characteristically free-living. The author has never found any reference to a gordiacean symbiote of an insect embryo in the literature.

The acanthocephalan worms are symbiotic during their entire life cycle, with no free-living phase. The writer found no examples of symbiotes of embryos from among this small group.

PART IV: THE ARTHROPODA:

Introduction

Before beginning this section the author wishes to make clear that he will only "scratch the surface"of the great mass of material regarding this group. The number of arthropod species is fabulous and any attempt to go through the lists of the known forms seeking out those that are symbiotic in other animals still in the embryonic stages would be futile. Arthropods are found in every kind of environment in every conceivable biologic relationship with other animals and plants. Much has been written about arthropods, especially the insects, Acarina, and Crustacea, and their habits. This thesis will deal primarily with the first two groups and some of their peculiar relationships to other plants and animals. This inclusion of plant symbiotes (a subject not specifically included in the title of the thesis) is not an attempt to consume the reader's time. Most of the work on the symbiotes of the two groups which include the insects, ticks and mites has concerned itself with the lower plant symbiotes and the symbiotes of obscure nature, namely, the rickettsiae and viruses. Also, methods of hereditary transmission of these symbiotes have been studied more carefully and thoroughly than in any other group of animals. Most of the space of the following pages will be devoted to a discussion of the different methods of transovarial transmission.

Parenthetically, it may be added that the impetus for

the great amount of work done on the symbiotes of the arthropods resulted fom the work of Smith and Kilbourne (1892) on the hereditary transmission of the Texas fever protozoan, Babesia bigemina (Smith and Kilbourne), by the tick, Boöphilus annulatus Say. Other infections have since been found that are transmitted by arthropods and pass from generation to generation through the eggs of the intermediate host. The ticks and mites seem to be very prone to transovarial transmission. For example, Texas fever, relapsing fever, rickettsial infections, tularemia, and tsutsugamushi fever are transmitted in this fashion from generation to generation. In the case of Texas fever and tsutsugamushi (transmitted by Trombicula akamushi (Brumpt), a mite) it is a necessary part of the mechanism of transmission. Not many insect-borne infections are hereditarily transmitted, but Trypanosoma cruzi in bugs and sandfly fever in Phlebotomus are exceptions. These examples are all of great importance to mankind, but many examples of transovarial transmission of symbiotic organisms - rickettsiae, and bacteria - are of importance only to the arthropod which harbors them.

In the Insecta, the best known arthropod class, those that are symbiotic during all or part of their life cycle are, for the most part, ectosymbiotes. All gradations of symbiosis exist between species of arthropods that are strictly symbiotic throughout their entire lives, e.g., itch mites and hair follicle mites, and species that are purely predatory and live

apart from their hosts except when feeding, like the mosquitoes. Close to the strict symbiote end of the series are the ixodid ticks and some adult fleas which leave their hosts intermittently. Further on the line are the bedbugs, triatomids, argasids, etc. which not only attend to their reproductive functions off the host, but also leave to digest their meals, symbiotes of the home rather than the host. Some arthropods such as certain mites, redbugs, botflies, screwworm flies, etc., are symbiotes only as larvae. The mites which are usually considered entirely symbiotic have many species which are symbiotic only during part of their life cycle and over half of the known species are not symbiotic at any stage of their life history. The most famous egg or embryo symbiotes belong to the Order Hymenoptera and the last portion of Part IV will consist of a discussion of this group.

Extracellular Bacteria

Glasgow (1914) was one of the first to study the bacteria found in the gut of insects and other arthropods. In his paper he brought out many interesting facts concerning the relationship. He observed that the bacteria from the different hosts species' ceca vary a great deal in their morphology though they are constant for each individual species of insect. He found that they ranged from minute coccus-like bacilli to huge spirochaete-like forms. He concluded that these bacteria must pass from generation to generation through the egg since they appear early in the alimentary tract of the developing embryo. This is one of the earliest recorded instances of the transovarial transmission of bacteria in insects. He believes that the chief function of these ceca-inhabiting bacteria is the inhibition of the development or the total exclusion of other foreign organisms. He assumes that the ceca merely provide a safe place for the multiplication of normal bacteria.

Several arthropods have solved the problem of transovarial transmission. Their solutions are modifications of a process by which the egg being oviposited is given a coating of the bacteria that are to form the intestinal flora of the young arthropod. Petri (1909-1910) described an outstanding example of the perpetuation of extracellular bacteria through successive generations. In his paper he discusses the case of _Xanthomonas_ (_Phytomonas_) _savastanoi_ (Smith), the cause of olive knot, and the non-pathogenic bacterium, _Ascobacterium_ _luteum_

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[illegible]

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Babes, which occur in the intestinal tract during all stages of development of the olive fly, Dacus oleae (Gmelin). Petri thinks that the bacteria help digest the great amounts of fatty oils the fly must take in, in order to extract enough nitrogenous substances for its development. The bacteria may be useful in breaking down the fats and releasing the nitrogen.

A longitudinal section of the ovipositor of the olive fly would show that the vagina and anal tract unite at their posterior end forming a common opening. In the wall of the anal tract near the point of union and opening into the lumen are peculiar saclike evaginations filled with bacteria. A longitudinal slit in the membrane which separates the anal tract from the oviduct lies immediately opposite the openings of the evaginations. As the eggs pass along the vagina, the surface of each egg is pressed through the slit, against the openings. The bacteria of the pockets are in this manner smeared over the surface of each egg, thence finding their way through the micropyle into the egg. The bacteria are then incorporated into the embryologic development of the insect.

In Tephritis heiseri (Frfld.) a similar process occurs except that the terminal end of the hindgut is differentiated into long-drawn-out channels which are narrowed in the direction of the cloacal aperture. These depressions are filled with bacteria which are applied to the eggs during oviposition. The freshly laid egg is covered with a layer of mucus in which the bacteria may multiply before they enter the egg through the

micropyle.

Parker and Spencer (1926) proved that *Pasteurella tularensis* (McCoy and Chapin) is transmitted from generation to generation and stage to stage in the Rocky Mountain wood tick, *Dermacentor andersoni* Stiles. This appears to have been the first recorded instance of the transovarial transmission of a known pathogenic bacterium by an arthropod. Philip and Jellison (1934) observed that a similar transovarial transmission takes place with this bacterium in *Dermacentor variabilis* (Say) but less frequently perhaps. Parker and Steinhaus (1943) report that *Salmonella enteritidis* Castellani and Chalmers may possibly be passed on occasionally through the egg of *Dermacentor andersoni* to the active stages of the next generation.

The next structure to be discussed has a slightly different functional advantage. In the Acanthosominae, from the ninth abdominal segment into the abdomen on each side projects an epidermal fold which consists, according to Steinhaus (1946), of numerous chitinous tubes filled with bacterial organisms. The "Beschmierorgan", as Rosenkranz calls it, coats the eggs with bacteria during the egg laying process. The bacteria do not enter the developing embryo but serve as food for the emerging larva.

Table VI lists some of those extracellular bacteria that manage to be transmitted from generation to generation through the egg by the methods outlined above.

Intracellular Bacteriumlike and Ricksettsialike Symbiotes of Arthropods

This section will deal mostly with a structure peculiar to certain groups of insects, the mycetome. Many workers have studied this organ but as yet its function is still uncertain. According to Aschner (1932) the mycetome has no function of its own directly related to the metabolism of the insect, its main role is that of providing a home for the symbiotes. It has been conjectured that, originally at least, the mycetomes were analagous to plant galls produced by the host as a response to irritation by a foreign inhabitant. The root nodules that harbor the plant symbiotes of the genus Rhizobium may be likened to some extent to the mycetome of insects. The various involution forms seen in insects are remindful of the life cycle of the Rhizobium. It is unfortunate though that attempts to grow the intracellular microorganisms of insects on culture media have not yet met with the success that has been had in the culturing of the plant symbiotes (Steinhaus, 1946).

The mycetome is that "organ" or structure, the cells of which, in many insects, harbor intracellular microorganisms or symbiotes. The name was first used by Sulc (1910) who also suggested the name mycetocytes for the individual cells making up the mycetome that are usually filled with the symbiotes. "Mycetocyte" (or bacteriocyte for cells harboring organisms definitely bacterial in nature) may also refer to single cells

containing symbiotes, even though they are not grouped together in a mycetome.

Leydig (1850), Huxley (1858), Metchnikoff (1866) and Balbiani (1869-1871) all studied the structure of the mycetome in the aphid but the conclusions they derived from their observations were erroneous. Witlaczil (1884) traced the origin of the aphid mycetome from the follicular epithelium. He also found a tunica propria enveloping the structure. Uichanco (1924) found that during the early stages of the aphid's development the follicular epithelium becomes infected with the symbiotes (by way of the nurse cells). The epithelium apparently harbors them in a dormant condition. As the egg forms in the adult, the symbiotes in the follicles come in contact with the yolk. This contact apparently stimulates the dormant symbiotes, bringing about their rapid multiplication and causing considerable swelling in the portion of the follicular epithelium adjoining the posterior pole. The symbiotes soon break through the thin epithelial cells and invade the posterior portion of the egg through the posterior opening in the periplasm. Thus the symbiotes are hereditarily transmitted. The symbiotes are intercepted in their migration toward the posterior pole by the vitellophages or "mycetoblasts". The syncytial mycetoblasts form a sublobular mass together with the cytoplasmic network, and the enclosed symbiotes. The new structure is the mycetome. There is much mitotic activity among the mycetoblasts and cellular differentiation appears.

By longitudinal division of the mass the mycetome becomes the size and shape seen in adult aphids. Henneguy (1904) postulated that the green, granular mass in the eggs of aphids consisted of microorganisms and Pierantoni (1911) and Sulc (1910) concluded, principally on the basis of morphologic characteristics (since the organisms could not be cultured), that the organisms were related to yeasts.

Klevenhausen (1927) discusses cases of mono-, di-, and trisymbiosis and the manner in which the eggs and embryo become infected with one, two, or three symbiotes.

The development of the mycetome in other insects is very similar. There is no essential difference between the mycetomes of the males and females. Some mycetomes harbor no symbiotes while other cells may sometimes contain numerous symbiotes.

Blochmann (1886) noticed that bacteria-like bodies were present in both the eggs and follicular membranes of ants and wasps. He suspected that these forms were bacteria. In further studies (1887, 1888) he expressed greater confidence in his belief as to the nature of the bodies, which he also found in the fat tissues and eggs of *Blatta orientalis* Linn. and *Blattella germanica* (Linn.). His proof that they were bacteria rested on their staining qualities, reactions to reagents, and their reproduction by fission. He tried unsuccessfully to cultivate the organisms in a beef-peptone-gelatin agar.

In some ants like *Camponotus* the symbiotes in the follicle give rise to an invasion of the egg plasma. How the symbiotes get from the intestinal epithelium to the developing eggs is not known with certainty. The symbiotes enter the follicular cells very early in the insect's development. From here they get to the egg cell and nearly fill it. Further development of the egg causes segregation of the primary mycetocytes. Small, angular cells develop which "ingest" the microorganisms. The primary mycetocytes deteriorate. These secondary mycetocytes are later included in the embryonic mid-intestinal wall cells. Much has been written on the processes involved in this transovarial transmission. Buchner (1930) did much to systematize and correlate the early available information. In *Oryzaephilus* and *Lyctus* (Coleoptera) and certain Anoplura the posterior pole of the egg is invaded shortly before the secretion of the chorion (Koch, 1936). In some pupiparous diptera and in *Glossina* which nourish the larva in the uterus, the symbiotes are transmitted through the milk glands.

Gier (1936) found mycetocytes in the body cavities of both male and female *Periplaneta americana* (Linn.). No symbiotes have ever been found within the testes themselves. In the ovaries, however, a layer of symbiotes is present around the larger oöcytes. The number of symbiotes between the oöcyte membrane and the follicle cells increases until there is a uniform layer two to three organisms thick. Before the egg is oviposited, the original oöcyte membrane breaks down and permits

the symbiotes to enter the cytoplasm. This introduces the organisms into the embryo. Accompanied by vitellophage-like nuclei, the microorganisms move in masses to the center of the yolk. As the embryo develops, a few organisms from this central mass move posteriorly between the yolk granules through the incomplete margins of the gut epithelium into the body cavity. From here most of them are taken up by the cells of the lateral lobes of the fat bodies while a few are caught between the cells of the developing ovaries. The symbiotes within the ovarioles lie dormant for several weeks and then they begin to rapidly multiply and spread over the surfaces of the enlarging oöcytes. Thus transovarial transmission is accomplished. Apparently the organisms in the eggs are always smaller than those in the adult. The behavior of the symbiotes is similar in all roaches though Buchner (1930) reports variations especially in the formation of the primary mycetome in the egg.

Mansour (1934a) discovered a similar process in certain bostrychid beetles (*Rhizopertha cominica* (Fabr.), *Simoxylon ceratoniae* and *Bostrychoplites* zickeli (Marseul)). The microorganisms from the mycetomes invade the testis lobes, multiply and mix with the sperm. Along with the sperm, they get to the bursa copulatrix of the female. From here they migrate through the micropyle of fully formed eggs during oviposition. How the bacteria get to the testes region is still unknown but

Koch (1931) thinks that they may leave the mycetomes through a rupture in the outer cover as they do in the females of Camponotus (see above). Experimental evidence (Glaser 1920; Aschner 1932, 1934; Aschner and Ries 1933) seems to show that the relationship here between symbiote and host is one of mutualism or true symbiosis.

Examples of Bacterium- and Rickettsia-like Symbiotes Arranged According to the Embryonic Arthropod Hosts

Acarina: Neither family of the ticks has members which possess a mycetome. In the ixodid ticks the symbiotes pass from the heavily invaded cells of the ovary to the developing oöcyte (Buchner, 1930). At first the symbiotes are gathered at either pole of the egg. Later, however, the symbiotes increase greatly in numbers, and as the storing of the yolk commences, a ball of symbiotes begins to form between the nucleus and the periphery like a secondary nucleus. Later, the mass breaks down to its individual components. A different situation exists in the argasid ticks. According to Mudrow (1932) these less pleomorphic symbiotes may not invade the first sex cells but are probably transferred from the Malpighian tubes to the ovarioles at a later stage. Reichenow (1922) studied the symbiotes of the family Dermanyssidae of the mites. Here the symbiotes remain enclosed in the yolk mass of the egg through the larval stage of the mite. When the host reaches the nymphal stage, the intestinal epithelium is formed, the yolk is absorbed and the mycetomes are formed.

Insecta:

Order Corrodentia: Sikora(1918, 1920) observed a small rickettsia-like organism on the stomach epithelium of the dust louse, Psocus, which is transmitted, he found, to the next generation through the egg.

Order Orthoptera: As a result of the investigations of Blochmann (1887), Mercier (1906, 1907), Buchner (1912), Fraenkel (1921) and Gier (1936) the life cycles of the symbiotes of roaches are fairly well known. Transovarial transmission in this group has already been discussed (page 43).

Order Homoptera: The aphids have already been discussed (page 42). Profft (1937) observed that in the family Adelgidae (conifer "pests") infection takes place by the accumulation of symbiotes between the follicle cells and the ova. They pass into each egg and into definite cells at the time of invagination of the embryo. In sexual females the entire symbiote population passes into the eggs. In addition to the ordinary mycetocytes, the jumping plant lice also transmit transovarially syncytial symbiotes. Both forms of symbiotes enter the eggs simultaneously and are taken up by the follicle cells at the posterior pole of the egg from which they pass into the egg itself. Upon the formation of the embryo's germinal streak, the two types of symbiotes separate and occupy different positions in the embryo. Buchner (1921) reports on the symbiotes of the ensign coccids. In invading the egg the symbiotes first arrange themselves in "tactical formation" between the follicular membrane and the anterior pole of the egg. The egg plasma then envelopes this mass of symbiotes and pulls it completely within itself. Transovarial transmission among the coccids has been summarized by Carter (1935). In Pseudococcus brevipes (Ckll.) and P. citri (Risso) the process is as follows: as the

ovaries develop, the contents of certain mycetocytes aggregate into small, very dense, and deeply staining bodies. These migrate from the mycetome and enter the egg at its junction with the nurse cells. Cowdry (1925) suspects that the rickettsia-like organism he found in the gut and Malpighian tubules of Magicicada septendecim (Linn.) is transmitted from generation to generation through the egg.

Order Hemiptera: Two genera in this group are known to transmit rickettsia-like organisms through their eggs. These are Triatoma rubrofasciata (DeG.) and the genus Ischnorrhynchus.

Order Coleoptera: Mention has been made of the situation in Lyctus linearis Goeze (see page 44.)

Order Diptera: In the family Pupipara which has been referred to previously (page 44) we have a few specific examples of transovarial transmission of intracellular symbiotes. Kligler and Aschner (1931) have found such transmission in Nycteribia kollari Frfld., N. biarticulata Herman, N. blasii Koll., and Pseudolynchia canariensis (Macq.)

Order Anoplura: Hooke and Schwammerdam in the seventeenth century had observed the mycetomes of lice. Other observers in the nineteenth and early twentieth century also studied them carefully. Florence (1924) found that the symbiotes shift location often, that is, as the animal grows from the egg they too move to new locations.

Order Mallophaga: The pigeon louse, Columbicola columbae (Linn.), passes its symbiotes transovarially. Ries (1931)

found that the symbiotes of the adults migrate individually from the mycetocytes up the ovariole and into a depression at the posterior end of the egg. Although the symbiotes are not as closely associated with the germ band as those in *Pediculus*, an anopluran studied by Aschner (1934), like the latter they are carried more deeply into the yolk with the invagination of the germ band. Yolk cells mingle with the mass of symbiotes, becoming the nucleus of the mycetocytes, which are surrounded by membranes. In the males the mycetocytes migrate into the developing fat bodies while in the females they pass into the ampullae of the reproductive organs. Many other examples of such associations are found in the literature.

Order Hymenoptera: Material relating to the genus *Camponotus* has been reviewed above (page 44).

Rickettsiae as Embryo-symbiotes

There may be some justification in including this group due to their obscure nature. Botanists, it seems, have not taken them into their fold and there is the possibility that with further research that they may be found to be animal in nature. Steinhaus (1946) defines a rickettsia as "any adequately studied organism which is usually, or at times, intimately associated with an arthropod, which is usually small, often pleomorphic, and gram negative, which in tissues stains lightly with most aniline dyes, and which is usually difficult to cultivate on cell-free media or which has not been so cultivated" - - a rather noncommittal definition. Many rickettsiae have been implicated in the causation of infection among animals and their arthropod vectors have quite often been thought to transmit the rickettsiae from generation to generation through the egg. For many rickettsiae such as R. prowazeki De Rocha-Lima (1916) which causes typhus this transovarial transmission in the flea and louse has never been proven.

There is indirect evidence that Rickettsia tsutsugamushi (Hayashi, 1920) is transmitted to the larvae of the mite vectors via the egg. Farner and Katsampas (1944) have noted the importance of this to the epidemiology of tsutsugamushi (flood) fever. The trombiculid larva, T. akamushi (Brumpt), takes only a single blood meal from a single host during its life cycle: therefore, following infection of a larva by feeding on an

infected host, the rickettsiae are not transmitted to another vertebrate host until the life cycle of the infected larva has been completed and a new generation of larvae have been produced, and then only if these larvae have received the organisms from the female through the eggs. Other redbugs are incriminated as vectors of similar infections in other oriental countries and even the United States where Bullis fever is suspected to be imported tsutsugamushi fever.

The rickettsia that causes sandfly fever was long suspected to get from generation to generation via the egg of the short-lived sandfly, Phlebotomus papatasi Scopoli, because the adult fly frequently sucks blood only once, whereas the disease can be transmitted by apparently unfed flies. Whittingham (1922) proved this transovarial transmission by producing sandfly fever by the bites of flies bred from infected parents.

Protective vaccines against typhus are being mass produced by a method introduced by Cox (1938). He found that the rickettsiae of typhus would grow luxuriantly in the yolk sac of developing chick embryos.

Some additional examples of transovarial transmission of rickettsiae in their arthropod vectors will be found in Table VII.

Yeasts and Fungi Associated with Insect Embryos

The generation to generation transmission of Saccharomyces in Stegobium paniceum (Linn.) and related insects is especially interesting. Under the wrinkled vagina of the female are two long, symbiote-filled, chitinous pockets shut off from the outside by two overlapping chitinous plates. Near the outside opening the pockets and the vagina unite. The vaginal pockets are filled during the first defecation of the young adult. Before the beetle leaves the puparium the mycetocytes migrate through the gut and anus to the ovipositor where they may get to the transmission organs. As was seen with certain bacterial symbiotes the eggs are smeared with the symbiotes. When the larva hatches it leaves the eggshell head first, consuming about one half of the shell in the process. The hitherto sterile larva now becomes infected with the symbiotes. Koch (1933) thinks that the symbiotes contribute to the nutrition of the insect. In other Coleoptera similar processes of transovarial infection occur. The yeast symbiotes of the aphids were discussed above in the section dealing with the formation of the mycetome in these insects. In the homopteran subfamily Diaspidinae the symbiotes leave the mycetocytes and apparently are carried through the blood stream to the ovarioles (Buchner 1921, 1930 and Rickter 1928). According to Shinji (1919) and Rickter (op. cit.) numerous other homoptera transmit Asterolicanium aurem (Bdv.) transovarially. The members of the

family Margorodidae manage to get into the eggs of their homopteran hosts by way of a canal in the peculiar "plug" associated with the maturing ovum (Buchner 1930). Iachardina lobata Chamb. and T. sylvestrii Mahd. (shellac producing scale insects) both have unidentified yeast symbiotes which Mahdihassen (1924-1935) found to be transmitted transovarially. Buchner (1930) describes the method of infection by Coccidiomyces dactylopii Buchner found in Pseudococcus citri (Risso). Here the symbiotes are taken in by a depression at the anterior pole of the egg.

A checklist of the North American entomogenous fungi has been compiled by Charles (1941). Because of their obvious plant nature, the fungi will not be treated in any detail. Petch (1925) isolated Spicaria javanica (one of the Fungi imperfecti) from the egg masses of Homona coffearia, a lepidopteran.

Some Viral and Spirochaetal Relationships with Embryonic Arthropods

Yellow fever virus has never been proven to pass through the egg to the next generation of *Aëdes aegypti* (Linn.) although Philip (1929) and others tried to observe it. The same is true of the dengue virus (Blanc and Caminopetros, 1929). In recent years other viruses pathogenic to animals have been found to penetrate the developing ova of arthropods and a listing of these will be found in Table IX. The only known instance of transovarial transmission of a plant virus is the transmission of *Oryza virus I* which causes a dwarf disease of rice in the leafhopper *Deltocephalus dorsalis* Motsch. This occurs only when the females harbor the virus; infected males do not transmit the agent to the progeny of noninfected females (Steinhaus 1946).

Hindle (1912) reported that when the spirochaetes of fowl septicemia, *Borrelia anserina*, are taken up by the tick, *Argas persicus* Oken, they invade the cells of the body, particularly those of the Malpighian tubules and the lumen of the gut, in which locations they break down into granules which continue to multiply. Some of these granules are supposed to enter the eggs and in this manner are transmitted to the next generation. Another Borrelia, *B. theileri*, which is found in the blood of cattle is transmitted by the tick vectors *Margaropus* decoloratus (koch) and *Rhipicephalus evertsi* Neum. In both cases the spirochaete is transmitted to the next generation via the egg.

Koch in East Africa and Dutton and Todd in the Belgian Congo simultaneously established (1905) that the relapsing fever spirochaetes are transmitted transovarially in ticks. Nicolle reports that the hereditary transmission of these spirochaetes occurs in some cases but is not common in lice.

Insects Symbiotic Within the Eggs of Other Insects and Spiders

The entomophagous insects about to be considered represent a polyphyletic series. Some are clearly derived from predators, animals of carnivorous habits that seek out and devour their prey; others are derived from scavengers and still others from phytophagous ancestors. Extreme specialization and convergence in both structure and development are commonly encountered throughout the entire series and this serves further to obscure the actual relationships between the numerous groups of insects concerned. The greater number of insect symbiotes are members of the Order Hymenoptera. Since all the primitive families of this group are phytophagous, feeding either in plant tissues or consuming the leaves, the symbiotic Hymenoptera must be derived directly from vegetarian ancestors. This does not include the symbiotic bees and some of the symbiotic wasps which have passed through a predatory stage in their development. Some symbiotes have evolved from scavengers. One species of fly is known to enter the burrow of a solitary wasp of the genus Sphex and deposit a freshly hatched larva on the caterpillar that has been placed therein as food for the wasp larva. The fly larva first destroys the wasp egg and then enters the paralyzed caterpillar. The host egg is carefully dispensed with to avoid any controversy later as to the ownership of the food.

The entomophagous insects fall into two different categories, the true, or "non-fatal" symbiotes and the "fatal"

symbiotes or parasitoids. By far the greater number of symbiotes belong to the category of parasitoids. These are symbiotes in the larval condition only and, unlike symbiotes belonging to the preceding category, their adult forms are active and non-symbiotic insects. They are relatively large in comparison with the size of their hosts which are almost always destroyed as a result of their activities. The vast majority of hosts are other insects, and these may be invaded while in the egg or later stages. The imagines, however, are very seldom attacked. The reason for this limitation of hosts is that most of the other invertebrates are marine, an environment not suitable for insects. Spiders' eggs are commonly infested with true internal symbiotes in the form of minute serphoid Hymenoptera of the genus Baeus and its allies. For the most part the spiders suffer more from predators than from egg symbiotes. One spider symbiote is Mantispa, a member of the Neuroptera, most of which are very active predators. The second instar Mantispa larva is typically a symbiotic type within the egg mass.

Girault (1907-1911) lists what he calls the "true" egg symbiotes known at that time. These symbiotes are insects that pass their entire life cycle (except as adults) within the egg of another insect. Predators are ignored in these papers as are symbiotes that oviposit on or into the egg, becoming fatal during some phase of postembryonic development. These he considered parasites of the larva or pupa as the case may be. Figures derived from Girault's findings will be found in Table XI.

The first and most important event in the life of any symbiote is the establishment of contact with its host. The most positive method of approach is the insertion of the egg directly into the host. The sharp stiletto-like ovipositor of the endosymbiotic female Hymenoptera is beautifully adapted to this purpose since it acts as a hypodermic needle. Through this, the very small flexible egg may be squeezed into the hemocoel of the host, sometimes into a particular structure such as a nerve ganglion, or into a host egg.

The passage of the egg through the very small lumen of the ovipositor has been carefully observed in a chalcid wasp, Habrocytus, by Fulton (1933). The tip of the egg is worked through the ovipositor by means of minute stylets, then the contents of the egg flow through the greatly constricted portion and the protoplasm accumulates within the host to be followed finally by the remainder of the rubber-like chorion that envelops the egg. To insure that only one symbiote will be deposited in the host and also to determine if the embryo is at the proper stage of development, the female hymenopteran usually probes with her ovipositor before ovipositing (Clausen, 1927). This cautiousness prevents the phenomenon of "superparasitism".

Since egg symbiotes are probably the most potent enemies of our insect pests they have been exploited by man to help in his fight for food and raw materials with insects. In the last half century many insect pests have been accidentally imported into our country and have caused untold damage to the economy

of the land. The ecological principal of the biotic potential of a species lies at the bottom of the problem. The ability of a species to multiply in a given time when freed from all environmental resistance is its biotic potential. The factors constituting environmental resistance are physical and biological. The main physical factor is climate while biological factors include symbiotes, predators, food, disease, and competition with other species. In the case of symbiotes and predators their capacity for discovering their hosts, in relation to the density of the latter, largely determines their efficacy as controlling agents. Thus, the greater the host density the easier it will be for natural enemies to discover them. Convincing evidence of the controlling influence of such agencies as symbiotes and predators in the native habitat is afforded in the case of insects that have assumed the status of major pests when accidentally introduced into lands they did not previously inhabit. In the new environment biological factors that were operating in the original habitat are wanting. In a number of instances of this kind, biological control has been achieved by the introduction of specific natural enemies whose activities have reduced the density of the population of the introduced insect to a degree in which the economic damage caused is negligible.

The specificity of the entomophagous symbiotes in selecting particular hosts is generally very great. There are

notable exceptions, however. For example, some of the most minute chalcid wasps belonging to the genus Irichogramma which develop within the eggs of larger insects exhibit a most extraordinary range. Pentarthron minutum (Riley) according to Girault (1911) maintains the lead as one of our most prominent, common, and widespread symbiotes of the eggs of insects. At that time it had been recorded from thirty or more hosts in North America and various other hymenopteran and lepidopteran hosts throughout the world. It is chiefly a symbiote of insect pests such as the codling moth, cotton bollworm, etc. More examples of insects with a wide range of hosts will be found in Table XII. Ordinarily all the symbiotic forms have a select clientele and do not form new associations readily.

A common chalcid symbiote of gypsy moth eggs is Anastatus bifasciatus Gir. The gypsy moth hibernates in the egg stage and the caterpillars hatch in the early spring. The Anastatus delay their emergence, however, until the eggs of the moths of the next generation have been laid in the late summer when they find fresh eggs in which to deposit their own eggs. This and similar symbiotes depend absolutely on the proper timing of their developmental cycle. Sometimes another acceptable host is available to fill the gap if one appears.

Crossman (1925) describes his experiments with Schedius kuvanae an insect egg-symbiote from Japan. It is normally a symbiote of Porthetria dispar (Linn.), the gypsy moth and the

brown tail moth, Euproctis chrysorrhoea Linn. This symbiote was liberated around Boston in the early 1920's in an attempt to control these two pests. It was found that Schedius bred from dead gypsy moth eggs were not so strong and robust as when bred on healthy gypsy moth eggs. Crossman also experimentally infected Hemerocampa Leucostigma (S&A), H. definita, Callosamia prometheae, Malacosoma americana (Fabr.), Hemileuca maia (Dru.), H. olivae Ckll., and Stilpnotia salicis (Linn.). Clausen (1927) reported on Anastatus albitarsis Ashm., a symbiote of Dictyoploca japonica. The larva of Anastatus semiflavidus Gahan prevents the formation of the embryonic larva of its host (Hemileuca olivae or H. nevadensis) and feeds exclusively upon the liquid contents of the host egg. This method of larval development is characteristic of the true egg symbiotes (Caffrey, 1927).

The Hymenoptera are truly remarkable for the highly evolved state that symbiosis has reached in the order; tens of thousands of species have acquired this habit (many in their host's egg and embryonic stages) and their larvae present special respiratory and other adaptations in accord with their modes of life. The adults too while mostly free-living have specialized adaptations for continuance of their species. Certain minute chalcids such as Polynema that dwell in the eggs of various aquatic insects, swim beneath the water by means of their wings. The ovipositor varies greatly in length among the symbiotic Hymenoptera and is longest in those species that have to

penetrate the wood of trees in order to reach their victims. Members of the Ichneumonidae have ovipositors three times the length of the insect's body. The injection of the symbiote's egg is frequently accompanied by a peculiar process of feeding by the female at the puncture made by the ovipositor. This diversion furnishes droplets of food at intervals during the period of egg laying and no doubt serves also as a stimulus that may have a psychological as well as nutritional basis. Another phenomenon associated with the symbiotic way of life is that of polyembryony which hastens the death of the host. Another odd fact about hymenopteran egg symbiotes is that there is usually much similarity between the symbiote and the host. Brues (1946) quips that this would remind one of the human symbiotes such as gangsters, etc. which are very similar morphologically to their hosts.

As an indication of the great number and distribution of species of hymenopterous egg symbiotes we have the figures compiled by Bakkendorf (1933) in his studies of the hymenopterous egg symbiotes chiefly of homopterous eggs in Denmark. He found that twenty seven different species representing four families were symbiotes in homopteran eggs. Table XII contains a list of some of the hymenopterous families which have members that are egg symbiotes of other arthropods.

Like the Hymenoptera, many Diptera are also furnished with a sharp and pointed or blade-like ovipositor that may be thrust directly into the body of the host. The only family of Diptera

that has members that are egg symbiotes is the family Bombyliidae. Species in this family attack principally the egg masses of grasshoppers (Clausen, 1940).

The Coleoptera, or beetles, form the largest order of insects, and although a great many are predators, almost none are symbiotes. The symbiotic forms include the members of two families, the Meloidae and Rhipiphoridae and a few scattered genera in several other families. Many Meloidae develop in the cells of solitary bees, their larvae first destroying the host egg and later consuming the stored food supply. Other meloids feed within the egg-masses of grasshoppers and are thus probably to be regarded as purely predatory. Zakhvatkin (1931) studied the coleopteran symbiotes of the egg pods of injurious locusts in Turkestan. Table XI contains some of his findings. This table shows a loss of host-specificity on the part of the symbiote. There were 21 symbiotes distributed through 9 host species.

Balduf (1933) feels it is questionable whether any of the Pyraustidae (Order Lepidoptera) are symbiotic in the eggs of other insects. Other scattered cases of symbiosis are found among certain moths, although most of these verge closely on predatism (for example caterpillars that feed within the egg masses of spiders).

The exclusively entomophagous order Strepsiptera which is also exclusively larviparous has no members that stylopize the

embryos of other insects. The larvae lie in wait for a nymphal or adult host to pass.

The Thysanaptera, Neuroptera (Mantispidae), Corrodentia, and Thysanura all contain members that are predaceous upon eggs of other insects or spiders. The hemipteran families Lygacidae and Myridae have become predaceous. The aquatic larvae of the Trichoptera are also predaceous as are adult Mecoptera and the plecopteran family Perlidae. The Odonata are predaceous during all stages while some of the Orthoptera are cannibals. All families of this group have some predaceous members while the Mantidae are all predators.

PART V EXPERIMENTAL INFECTION OF CHICK EMBRYOS WITH HETERAKIS EGGS

Heterakis gallinae (Gmelin, 1790) Freeborn, 1923 is the common nematode found in the caeca of domestic fowl throughout the world. It also dwells in the small intestine and colon but it is the only member of the genus Heterakis that is found in the caeca of its host. Cram (1927) lists 35 species of avian hosts (in 20 genera) but the common poultry species harbor this symbiote most often. Its incidence varies throughout the world but it has been computed by Cram that the incidence in chickens is 75.2 per cent. The symbiote does not seem to have any pathogenic effect upon its hosts, as evidence the fact that a caecum containing 700 of the symbiotes appeared normal in spite of such a heavy infection (Clapham, 1933). The lumen was never occluded. Riley and James (1921) also studied the effects of large infections upon the health of the host and they too could not find any pathogenic effects.

The adult worm is small, whitish, and rather opaque with the anterior end usually flexed dorsally from the region of the esophagus. The rest of the body lies straight. It is covered with a cuticle which is traversed by fine transverse striations about 3 μ apart. The cuticle is expanded laterally into two flanges extending from shortly behind the mouth nearly to the end of the body. This cuticle is smooth. The esophagus is filariform and the cuticular masticatory apparatus consists of three well-marked dentate valves in the interlabial position.

The adult male Heterakis measures 7 to 12 millimeters while the female measures 8 to 16 millimeters and is bulkier. The anterior end of the female is more frequently flexed than the male.

The eggs are ovoid and bluntly rounded at both poles, and measure from 70 μ to 80 μ by 38 μ to 44 μ with an average of 73 μ by 42 μ. The eggs are covered with a thick hyaline shell 5 u thick. Under the shell is a somewhat irregular membrane pierced by a clear lenticular space at one pole. The shell is secreted in the terminal portions of the uterus since the eggs in the inner lumen of the uterus lack shells. No segmentation occurs in the caeca of the host. (Clapham (1933) thinks that this is due to a lack of oxygen here.) The eggs pass out in the feces while in the single celled stage.

The development of the egg to the infective stage occurs in from two weeks to seventeen days. The temperature range for development is 20 to 30 degrees Centigrade with an optimum of about 26 degrees. The eggs can be cultivated in Petri dishes especially if the dishes are well aerated. Various media can be used for their development including dilute solutions of acids and disinfectants. Clapham found that 1 per cent formalin cultures were viable after seven months while Tyzzer (1934) reports that the infective eggs can overwinter in the soil of New England.

The first cell division occurs within 24 hours of the exit from the host body and a morula is formed by the third day.

A large number of blastomeres are produced. These blastomeres tend to be smaller at one pole than at the other. At the end of a week a depression forms between these two types of cells. This deepens and the embryo grows terminally, thus taking on a distinctly vermiform appearance.

The slightly motile first stage embryo is formed in the shell but is quickly superseded by the second stage embryo. Its esophagus is double bulbed (rhabditiform). The first stage embryo moults and gives rise to the second stage embryo which remains enclosed in the first stage sheath which it loses only when ingested by the host and the egg hatches.

The infective stage is this second stage embryo. The worm larva is seen coiled two or three times within the shell and it measures 235 by 20 μ. A large number of these infective eggs are carried mechanically through the gut and pass out unhatched in the feces of the poultry which ingest the eggs. The others hatch after an hour or two in the first quarter of the intestine. The egg shell splits longitutinally from the micropyle in the inner shell membrane. The embryos pass slowly down the intestine to the caeca where they undergo their second moult within 24 hours.

The third stage larva measures 640 by 45 μ and moults to form the fourth stage larva 96 hours after the egg is ingested. The fourth stage larva measures 3 to 4 millimeters. The sexes can be distinguished at 8 days. The worm is mature by 22 days and eggs are passed in the feces two days later.

Thus it is seen that the life cycle is direct and involves no intermediate host. The complete cycle was first worked out by Clapham (1933). A period of development outside the host is necessary while the eggs become infective. After being ingested by the host, the larvae hatch, pass directly to the caecum and mature there in its lumen. Experiments by Clapham show that no migration occurs within the host.

The Relationship of Heterakis gallinae to Blackhead Infection

Blackhead, a disease which often decimates poultry flocks especially flocks of turkeys, is caused by a protozoan Histomonas meleagridis (Smith 1895). This protozoan invades the tissues of the host causing lesions which ultimately lead to the death of the bird. The pathogen is variously transmitted from one bird to another. Barnyard soil containing embryonated eggs of Heterakis is the most common method of transfer in the natural state. Material taken from the lesions which contain the protozoans when inoculated directly into susceptible birds causes a typical infection. Thirdly, the caecal contents of an infected bird which is a carrier also contain the protozoan and such material can be fed directly to a susceptible bird.

The infection is progressive in young birds, therefore, it is almost always fatal. There is wide variation in its pathogenicity. It is mild in chickens and extremely pathogenic for turkeys. In the chicken the protozoan is quickly expelled from the tissues but establishes itself in the caecal contents. Here it remains and multiplies and the chicken thus becomes a carrier.

Graybill and Smith (1920) discovered that the feeding of embryonated Heterakis eggs caused typical blackhead infections. Many attempts have been made to see the protozoan in the eggs of the worm but all have been unsuccessful. The protozoan has been found, however, in the gut of 10 day old larval worms (Tyzzer, 1934). This is the only intestinal worm that has been

incriminated as a vector of an infectious disease.

As the result of his studies on the experimental infection of chicks and turkey poults with blackhead by the ingestion of embryonated Heterakis eggs, Tyzzer (1934) found that 70 per cent of the turkeys acquired the disease while only 55 per cent of the chicks were susceptible. He mentioned that not all ova carry blackhead infection. For an ovum to carry the protozoan the hen harboring the worm must either have had the disease and become a carrier or be currently infected with the protozoan. The ingestion of male Heterakis or non-embryonated eggs, Tyzzer found, would not produce blackhead infections.

Schlotthauer and Essex (1931) reported maternal transmission of Histomonas in birds. They noticed "blackhead" infections in week old poults. Tyzzer (1934) said it is doubtful if such occurs and he gave evidence for his contention by showing that Histomonas will die in an albumen medium at 38 degrees Centigrade.

Methods Utilized in the Experimental Infection of Chick Embryos with Heterakis gallinae

The *Heterakis* eggs used in the following experiments were obtained from the feces of a hen which came from a flock which had had an epidemic of blackhead a half year previously. The fertile eggs were obtained from the well kept flock of a poultry farmer.

The helminth eggs were isolated by the zinc sulfate (specific gravity 1.18) flotation technique from fresh feces. The eggs were cultured in a variety of media including ordinary tap water, boiled and aerated pond water, and 1.5 per cent nitric acid (after a suggestion by Tyzzer, 1934). They were kept in covered Petri dishes to avoid excessive evaporation and atmospheric bacterial contamination. The lack of aeration did not seem to impede development. All cultures used in inoculating the chick embryos were more than seventeen days old which is the time it takes for the worm larva to have reached the infective stage. Also, all cultures that were inoculated into the chick's yolk sac were tested for the presence of bacterial contamination and all were found relatively free from bacteria (see Table XIII). A few of the cultures were placed in an incubator set at 37 degrees Centigrade. These failed to develop and were discarded (Clapham (1933) determined that the optimum temperature for growth was 26 degrees).

The number of viable eggs introduced into the chick was computed approximately by determining the number of completely

embryonated eggs in a known quantity of medium and multiplying by the total amount of medium This was done by introducing just enough of the concentrated suspension to fill a space of known volume (0.02 cc.) between a 7/8 inch square coverslip and slide. By reading the whole area beneath the coverslip the total number of eggs in that volume was found. The approximate numbers of eggs introduced into the chick embryos will be found in Table XIV.

To obtain the Heterakis eggs for inoculation the contents of the culture plate were centrifuged and the sediment was washed with 1.5 per cent nitric acid (in order to kill any bacteria present), the suspension was centrifuged and the sediment was washed with distilled water. Another centrifugation concentrated the eggs. The eggs were then placed in sterile test tubes and diluted with less than 1 cc. of warm sterile saline. By the use of an hypodermic syringe which was computed in hundredths of a cubic centimeter the amount of suspension introduced into the chick could be accurately determined. In all, 13 fertile and 6 dead embryonated eggs were inoculated. The first two developing eggs were exposed to view under nearly aseptic conditions and had windows inserted in their shells. Since the mouth in the 15 to 19 day old chick is hidden under the wings and the raised legs it was impossible to introduce the helminth eggs directly into it. The yolk sac (later to become part of the intestine and whose contents are completely absorbed) was inoculated since it was easy to find and could be

located without inserting a window in the shell. It was thought, therefore, that any material introduced there would ultimately get to the intestine.

In penetrating the shell of the egg a sterile high speed drill was used. Care was taken to avoid damage to the egg membrane since hemorrhage is likely to result. The hole was always bored in the same location because it had been observed that the yolk sac lay just underneath it. A blunt hypodermic needle of heavy bore was used to direct the saline suspension of eggs into the yolk sac. To seal the egg a sterile piece of cellophane one half centimeter square and wet with alcohol was placed over the puncture and affixed with Scotch tape. With such a seal the eggs could be periodically turned over to prevent the chick membranes from adhering to one another. Also, the size of the hole and the aseptic conditions under which it was made precluded the possibility that the egg would become infected with spores of fungi.

Of the 13 fertile eggs inoculated only 3 hatched and survived and these were kept isolated from one another in a specially constructed brooder which was heated by a hundred watt bulb. The unhatched chicks and the embryos which had died before inoculation were autopsied by opening up their visceral cavity and observing the gross appearance of the organs. The alimentary canal from the gizzard to the rectum was removed and cut open and its contents were scraped into saline as were the contents of the yolk sac. Direct smears of the suspension were

made and a portion of the suspension was centrifuged, the supernatant liquid poured off and zinc sulfate added to the solid in order to raise any eggs or larval worms that may have been present. The results of these autopsies will be found in Table XIV.

In order to determine if the surviving chicks had acquired the infection periodic fecal examinations using the zinc sulfate flotation technique were made. Results of these examinations will be found in Table XIV.

Autopsies performed upon the three surviving chicks showed no worms in the intestine. The intestine from gizzard to anus was removed, opened under water, and its surface scraped but no worms could be detected. A small pedunculate nodule was found in the upper small intestine of Chick No. 11. Upon microscopic study the contents of this nodule revealed no worms or eggs. The other two intestines appeared normal except that that of Chick No. 14 was much shorter and undeveloped as was the whole body of the stunted chick. Chick No. 11 was normal except for a malformed acetabulum of the right side of the pelvis which prevented it from walking.

It would seem from the results of the above experiment that it is not possible for a fowl egg to be infected by Heterakis gallinae. Of the nineteen autopsies performed worms were found only in two. Both worms observed were larvae of very small size. Only one autopsy revealed eggs of the worm. Both eggs were fully embryonated but had failed to hatch. The

problems are why had these failed to hatch and what happened to the other Heterakis eggs injected into the embryos. A possible explanation is that not all the eggs got to the yolk sac and, therefore, were lost in the fluids and membranes surrounding the chick. In such an environment the larvae probably would not hatch. If the larval worms penetrated to the intestine and caeca, the apparent lack of food and oxygen in these localities might have caused the death and consequent shedding in the mecomium of those that survived. The meconium was not tested for the presence of worms.

The death of the unhatched chicks may have been brought about by many factors any of which would have been sufficient to cause death. The eggs were incubated in an infiltrating oven which has very poor circulation of air. The moisture of the air, however, was adequate, i.e. the air was always saturated. Vibrations set up when drilling and the introduction of a large hypodermic needle, a comparatively large amount of fluid, and other necessary rough handling may have caused death. Since the inoculation of the embryo was performed under nearly aseptic conditions the possibility of pathogens causing death is slim. The worms themselves may have caused death but their absence in subsequent tests seems to rule out this possibility. Furthermore, tests (see above) have shown that these worms are not pathogenic for chickens. Typical "blackhead" infections in the dead chicks were not seen and so the presence of the pathogen,

Histomonas meleagridis, is eliminated. Reference to Table XIV will show that seven of the chicks died just before hatching and it was noticed that all four that did hatch had trouble penetrating the shell. Their weak condition might be attributed to the poor circulation of air in their "incubator".

All results gathered during this experiment will be found in Tables XIII and XIV.

TABLE I

PROTOZOA FOUND IN EMBRYONIC TISSUES OF VARIOUS ANIMALS

SYMBIOTE	HOST
Order Dinoflagellata:	
Orchitosoma parasiticum Chatton	ovary and testis and ducts of *Paracalanus parvus*
Chytriodinium roseum Chatton	eggs of pelagic crustaceans in Bay of Naples
C. affine	"
C. parasiticum	"
Trypanodinium ovicola	*Oithona* or *Clytemnestra* egg sacs at Banyuls-sur Mer
Olpidium	seen often in various aquatic invertebrates' eggs
Blastulidium	"
Order Rhizomastigina:	
Histomonas meleagridis	*Heterakis gallinae*
Order Protomonadina:	
Genus *Cryptobia* Leidy	reproductive organs of molluscs and other invertebrates
Crithidia hyalommae O'Farrell	*Hyalomma aegyptium*
Leptomonas jaculum	*Nepa cinerea* (water scorpion)
some monomorphic trypanosomes	chorio-allantoic fluid of chick (experimentally)

TABLE I (cont'd)

SYMBIOTE	HOST
Trypanosoma cruzi	chick embryo tissue culture, dogs, and one human case reported (Mazza, 1939)
Trypanosoma gambiense	chick embryos (artificial)
Trypanosoma brucei	" " "
T. rhodesiense	" " "
Order Polymastigina:	
Trichomonas foetus	bovine embryos
Trichomonas vaginalis	human (?) foetus
Genus *Hexamita*	*Deropristis inflata* Hunninen and Wichterman (1938)
Order Gregarinida:	
Genus *Monocystis*	seminal vesicles of annelids
Genus *Nematocystis*	" " " "
Genus *Rhynchocystis*	seminal vesicles of *Lumbricus*
Family Zygocystidae	seminal vesicles of oligochaetes
Family Stomatophoridae	seminal vesicles of *Pheretima*
Gonospora minchini	eggs of *Arenicola ecaudata*
Order Coccidia:	
Caryotropha mesnili	*Polymnia* (polychaete); cysts extruded with reproductive cells of host

TABLE I (cont'd)

SYMBIOTE	HOST
Karyolysus lacertarum	Liponyssus saurarum (mite) sporogony; lizard, *Lacerta muralis* is the final host.
Order Haemosporidia:	
Babesia bigemina	*Boophilus annulatus*
Babesia canis	*Rhipicephalus sanguineus*, *Dermacentor reticularis* and *Haemaphysalis leachi*
Anaplasmata:	
Anaplasma centrale	*Boophilus annulatus*
Anaplasma marginale	B. annulatus, *Dermacentor occidentalis* and *Ixodes ricinus*
Order Microsporidia: (see TABLE II)	
Order Myxosporidia:	
Wardia ovinocua	ovary of *Lepomis humilis*
Order Holotricha:	
Espejoia mucicola	gelatinous envelope of the eggs of certain insects and molluscs
Order Peritricha:	
Genus *Lichnophora*	*Crepidula* egg ectoparasite

TABLE II

MICROSPORIDIA OF THE GONADS AND EMBRYONIC TISSUES OF OTHER ANIMALS

HOST	SEAT OF INFECTION	MICROSPORIDIAN
Taenia bacillaris	gonads, ova	Gen.inc.*helminthophthorus*
T. denticulata	gonads, ova	Gen.inc.*helminthophthorum*
T. expansa	" "	"
Plumatella fungosa (Bryozoa)	testis	*Nosema bryozoides*
Ascaris mystax (from gut of cat)	female genital organ	*Nosema mystacis*
" "	gonads, ova	Gen.inc.*helminthophthorum*
Limnodrilus spp. (annelid)	spermatocyte	*Mrazekia caudata*
Ceriodaphnia fuscus	gonads	*Nosema cyclopis*
Crab	ova	*Thelohania maenadis* (Perez, 1906)
Podura aquatica	gonads	Gen.inc. *thysanurae*
Potamanthus (?) sp. nymph	gonads	Gen. & sp.inc. (Pfeiffer)
Ctenocephalus canis	ovary	*Nosema ctenocephali*
C. felis	"	" "
Anopheles maculipennis	ova	Gen. & sp. inc. (Christophers, 1901)
Anopheles sp. (adult)	ova	Gen. & sp. inc. (Christophers, 1901)

TABLE II (cont'd)

HOST	SEAT OF INFECTION	MICROSPORIDIAN
Anopheles spp.	ova	Gen. & sp.inc.Grassi
Aëdes aegypti	ova	Plistophora stegomyiae
Drosophila confusa	ova	Octosporea muscae-domesticae
D. plurilineata	ova	"
Musca domestica	germ cells	"
Bombyx mori	all tissues	Nosema bombycis
Pieris brassicae	ova	Perezia mesnili
" "	ova	P. legeri
Pyrausta nubialis	ova	P. pyraustae
Abramis brama x Leucistis rutilis (a fish)	ovary	Plistophora elegans
Alburnus mirandella	ovary, ova	P. mirandella
Barbus barbus	testis	P. longifilis
B. flaviatus	ovary	Gen. & sp.inc. Keysellitz
Coregonus exiguus bondella	ova	Thelohania ovicola
Sciaena australis	ovary	Plistophora sciaenae

TABLE III

SOME TREMATODA TRANSMITTED HEREDITARILY BY ANIMALS

SYMBIOTE	HOST
Monogenea:	
Gyrodactylus elegans	*Gyrodactylus elegans* (Chinese puzzle box phenomenon)
Udonella caligorum	Caligus spp. (Copepoda) egg sac hyperparasitism
Digenea: Family Plagiorchidae:	
Plagiorchis arcuatus	oviducts of fowl in Russia and Germany
Distoma ovatum	fowl egg
Prosthogonimus ovatus	oviduct of fowl, goose and various wild birds. Causes abnormalities of the eggs.
Prosthogonimus cuneatus	"
Prosthogonimus pellucidus	fowl and curlew oviducts
P. intercallandus	oviduct of fowl (fatal) also causes defective eggs
Diplodiscus temperatus cercariae	*Rana clamitans*
Family Schistosomatidae:	
Schistosoma haematobium	human foetus
S. japonicum	human foetus
S. mansoni	human foetus
S. bovis	cattle

TABLE III (cont'd)

SYMBIOTE	HOST
Schistosoma spindale	cattle
S. douthitti	muskrats and North American rodents
Bilharziella polonica	wild and domesticated ducks
Examples from other families:	
Ptychogonimus megastroma larvae	tubules of testes, vas deferens and coelom near ovary of *Brachyura* (Crustacea)
Fasciola hepatica	sheep, goat, cattle, other ruminants, pig, hare, rabbit, beaver, copyu, elephant, horse, dog, cat, kangaroo, and man.
Cercaria macrocerca	Sphaerium corneum (snail)
Cercaria echinatoides	Paludina vivipara (gonads)
Metacercaria capricosa	gonads of *Ophiothrix fragilis* and *Ophiura albida* (Ophiuroids)
Cercaria hemasthela secunda	gonads of *Littorina littorea* (a snail)
Cercaria lophocerca	"

TABLE IV

TREMATODE PARASITES OF THE BURSA FABRICII OF EUROPEAN BIRDS
(from Dawes, 1946)

SYMBIOTE	HOST
Prosthogonimus ovatus	carrion crow, rook, house sparrow, and coot
P. cuneatus	same as above
P. anatimus	domestic duck
P. rudolphii	" "
P. skrjabini	" "
Schistogonimus rarus	" " coot
Gymnophallus bursicola	common skoter
G. dapsilis	" "
Cotylurus variegatus	black-throated diver
C. erraticus	" " "
Apopharynx bolodes	coot
Parorchis acanthus	herring gull, common gull
Leucochloridium macrostomum	carrion crow
Catatropis verrucosa	common skoter

TABLE V

CESTODES AND NEMATODES FOUND OR SUSPECTED OF BEING TRANSMITTED HEREDITARILY

SYMBIOTE	HOST
CESTODA:	
Houttuynia struthionis	ostrich (?)
Taenia saginata	Calves (suspected)
Sparganum of *Diphyllobothrium latum*	roe of infected fish (possibly)
Sparganum proliferum	human (?) found in all tissues except bone
NEMATODA:	
Ascaris lumbricoides	man, certain large apes, and pig.
Ascaris inflexis	fowl
Toxacara canis	dogs and foxes
Trichinella spiralis	guinea pigs (experimental)
Genus *Ancylostoma*	dogs and foxes
Uncinaria stenocephala	dogs and foxes
Dictyocaulus filaria larva	sheep, goats, and some wild ruminants
Habronema megastroma	*Musca domestica* (xenodiagnosis)
Enterobius vermicularis	human (fallopian tubules)

TABLE VI

SPECIFIC BACTERIA ASSOCIATED WITH INSECTS AND TRANSMITTED TRANSOVARIALLY

SYMBIOTE	HOST
Corynebacterium ovis Bergey et al.	*Dermacentor albipectus* observed by Humphreys and Gibbon 1942.
Bacillus similcarbonis	*Melolontha vulgaris* and other flesh eating flies' egg envelope---Cao,1906.
Flavobacterium rheney (Chester) Bergey et al.	*Diaphemero femorata* (walkingstick) eggs Steinhaus, 1946.
Salmonella enteritidis (Gaertner) Castellani and Chalmers	*Dermacentor andersoni* (experimental) Parker and Steinhaus,1943.
Pasteurella tularensis	*Dermacentor andersoni* Parker and Spencer, 1926.

TABLE VII

SOME ADDITIONAL EXAMPLES OF TRANSOVARIAL TRANSMISSION OF RICKETTSIAE IN ARTHROPODS

SYMBIOTE	HOST (intermediate)	DISEASE	REPORTED BY
Rickettsia burneti	*Dermacentor andersoni*	Queensland ("Q") fever	Parker and Davis(1938)
Rickettsia canis	*Rhipicephalus sanguineus*		Donatien and Lestoquard (1935)
R. melophagi	*Melophagus ovinus*		Steinhaus (1946)
R. lectularia	*Cimex lectularius*		Arkwright, Atkins and Bacot (1921)
R. dermacentrophila	*D. andersoni*		Steinhaus (1942)
Wohlbachia pipiensis	*Culex pipiens*		Hertig(1936)
Dermacentroxenus rickettsi	*D. andersoni* *D. variabilis* *Amblyomma americanum* *Ixodes dentatus* *Haemophysalis leporis-palustris*	Rocky Mountain Spotted Fever	much literature
Dermacentroxenus conori	*R. sanguineus*	Kenya typhus(fievre boutonneuse)	Brumpt (1932)

TABLE VIII

ADDITIONAL EXAMPLES OF YEASTS FOUND TO BE TRANSMITTED TRANSOVARIALLY IN SOME INSECTS

SYMBIOTE *	HOST	OBSERVED BY
Lecanicola parasitica	_Coccus hesperidum_	Lindner (1895)
L. contei	_Saissetia hemisphaerica_	Conte & Faucheron (1907)
L. saissetiae	_Saissetia oleae_	Granovsky (1929)
Coccidiomyces dactylopii	_Pseudococcus citri_	Buchner (1930)
Icermyces pierantonii	_Icerya purchasi_	Buchner (1912)
Torula lecanii corni	_Lecanium corni_	Benedek and Specht (1933)

* The suffix -cola refers to those forms living free in the hemolymph or connective fat tissue. Suffix -myces refers to mycetome inhabiting forms (Brain, 1923).

TABLE IX

VIRUSES TRANSMITTED TRANSOVARIALLY BY ARTHROPODS

VIRUS	HOST	REPORTED BY
non-polyhedral "wilt" disease (pseudo-grasserie I)	*Euxoa segetum* (cutworm)	Paillot (1936)
western type of equine encephalitis virus	*Dermacentor andersoni*	Syverton and Berry (1941) Blattner and Heys (1944)
Russian spring-summer encephalitis virus	*Ixodes persulcatus*	Chumakow and Seitlenock(1940)
lymphocytic choriomeningitis virus	*Dermacentor andersoni*	Shaughnessy and Milzer (1939)
lymphocytic choriomeningitis virus	*Cimex lectularius*	Milzer (1942)

TABLE X

TOTAL NUMBERS OF INSECT EGG SYMBIOTES AND THEIR HOSTS COMPILED BY GIRAULT (1907-1911, 1914)

(For the names of the hosts and symbiotes the reader is referred to the papers above)

HOST ORDER	HOSTS FOUND IN EUROPE, AFRICA, AUSTRALIA AND ASIA		HOSTS FOUND IN NORTH AND SOUTH AMERICA	
	Hosts	Symbiotes	Hosts	Symbiotes
Coleoptera	7	8	15	16
Diptera	3	3	1	1
Hemiptera	19	20	39	59*
Hymenoptera	*	*	11	14*
Lepidoptera	65	72#	6	88*
Neuroptera	2	2	2	3
Orthoptera	6	10	34	49*
Odonata	-	-	2	5
Platyptera	1	2	1	1

many have more than one host.

* some questionable.

TABLE XI

SOME COLEOPTERAN SYMBIOTES OF LOCUST EGG PODS IN TURKESTAN

(after Zakhvatkin, 1931)

SYMBIOTE	HOST
Epicauta erythrocephala, Pall.	Calliptamus italicus L.
" "	Locusta migratoria L.
" "	Schistocerca gregaria Forsk.
Mylabris frovoli, Germ.	Dociostauris macrocannus Thnb.
" "	D. kraussi Ing.
" "	Calliptamus italicus
" "	C. turanicus Tarb.
Mylabris deserta Sum.	Dociostaurus macrocannus
" "	D. kraussi
" "	Rambouriella turcomana F.W.

TABLE XII

HYMENOPTERAN FAMILIES SOME OF WHOSE MEMBERS ARE SYMBIOTIC WITHIN THE EGGS OF OTHER INSECTS

FAMILY	SUBFAMILY	SOURCE
Braconidae		
	Cheloninae	Vance (1932)
		Cox (1932)
	Triaspinae	Clausen (1940)
	Macrocentrinae	Clausen (1940)
	Microgasterinae	Zorin (1930)
	Alysiinae	Clausen (1940)
Ichneumonidae		
	Cryptinae	Clausen (1940)
		Janvier (1933)
		Kelly (1914)
Trichogrammatidae		Taylor (1937)
		Clausen (1940)
		Bakkendorf(1933)
Eulophidae		Clausen (1940)
Aphelinidae		Clausen (1940)
Encyrtidae		Clausen (1940)
Eupelmidae		Clausen (1940)
Callimomidae		Clausen (1940)
Platygasteridae		Clausen (1940)
Scelionidae		much work
Serphidae		Bakkendorf(1933)
		Hendriksen(1918)
Ibaliidae		Chrystal (1930)
Myrmaridae		Bakkendorf(1933)
Callimomidae		Williams (1914)
Eurytomidae		Smith (1930)
Chalcididae		Bakkendorf(1933)

TABLE XIII

EXPERIMENTAL DATA

Heterakis culture	Culture Medium	Temperature of culture ($^{\circ}$C.)	Bacterial Cultures (agar pour plates)		
			24 hours	42 hours	70 hours
1	tap water	22	negative	negative	negative
2	pond "	22	neg.	neg.	neg.
3	" "	22	5 colonies	5 colonies	5 colonies
4	" "	22	5 colonies	5 colonies	5 colonies
5	" "	38	neg.	neg.	neg.
6	" "	22	1 colony	1 colony	1 colony
7	" "	38	3 colonies	13 colonies	13 col.
8	" "	22	neg.	2 sm. col.	2 small colonies
9	" "	37	neg.	neg.	neg.
10	1.5% HNO_3	20	neg.	neg.	neg.
11	" "	20	neg.	neg.	neg.
12	" "	20	neg.	neg.	neg.
13	distH_2O	20	surface film	surface film	surface film
14	HNO_3	20	neg	1 colony	1 colony
15	dist.H_2O	20	neg.	10 small colonies	10 small colonies
16	HNO_3	20	1 colony	1 large colony	1 large colony
17	HNO_3	20	neg.	neg.	neg.

ratio of non-embryonated to embryonated eggs	Remarks
1:5	-
1:8	-
none observed	culture discarded
1:10	-
13:0	culture discarded
1:5	1 worm hatched
6:1	culture discarded
0:15	1 worm hatched
4:1	culture discarded
0:30	-
0:25	-
1:3	-
3:5	-
0:3	3 worms had hatched
4:16	-
none observed	culture discarded
	-

TABLE XIV

EXPERIMENTAL DATA (cont'd)

Chick Injected	Culture Used	Date of Injection	Volume of Suspension Injected (cc.)	Approximate No. of Worm Eggs Injected	Fecal Examinations		
					10 days	15 days	17 days
1	1	4/5/49	.25	43	-	-	-
2	2	"	.65	68	-	-	-
3	4	4/6/49	.26	80	-	-	-
4	6	"	.35	51	-	-	-
5	8	"	.80	128	-	-	-
6	10	"	.75	90	neg.	-	neg.
7	10	"	.50	65	-	-	-
8	10	"	.75	90	-	-	-
9	11	"	.50	150	-	-	-
10	12	"	.30	25	-	-	-
11	13	4/8/49	.65	42	neg.	neg.	neg.
12	14	"	.80	25	-	-	-
13	15	"	.60	48	-	-	-
14	15	"	.70	42	neg.	neg.	neg.
15	17	"	.80	76	-	-	-
16	17	"	.40	48	-	-	-
17	17	"	.10	12	-	-	-
18	saline	"	.80	-	-	-	-
19	saline	"	.80	-	-	-	-

AUTOPSY RESULTS

Age of Embryo * (days)	Direct smear of gut contents	flotation technique	Remarks
20	negative	negative	
21	neg.	neg.	liver had lesions (?)
16	neg.	neg.	
20	neg.	1 larval worm	
7	neg.	neg.	results non-conclusive
hatched (normal)	-	-	no worms found, gut normal, yolk sac not completely absorbed
4	neg.	neg.	embryo mostly resorbed.
16	neg.	neg.	yolk solid with peculiar odor
died in incubator	neg.	neg.	yolk greenish in color
21	neg	neg.	yolk hardened
hatched (Ill)	*	-	no worms found. Small nodule in upper sm. intestine
18	neg.	neg.	gizzard had bloody contents
7-10	neg.	neg.	results non-conclusive
hatched (ill)	*	-	no worms found, gut normal, yolk sac not fully absorbed
4-5	neg.	neg.	partly resorbed. Yolk hardened. Results non-conclusive
5-7	neg.	neg.	results non-conclusive
20	2 eggs 1 larva	neg.	
7	neg.	-	results non-conclusive
19	neg.	-	results non-conclusive

* Chicks supposed to hatch on April 11.

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Abstract of Thesis

Symbiotes of animal embryos are uncommon because of the defenses of the animal embryo such as shells, secretions, isolation, etc. Not all symbiotes of embryos were mentioned because of the difficulties involved in finding material pertaining to some. Certain terms used in the thesis were defined at length. The word symbiote is preferred to the word parasite.

The subject matter of the thesis was broken up into three sections, the Protozoa, helminths and the arthropods, since these three animal groups contain most of the symbiotic forms. Included under the term embryo-symbiotes are the symbiotes of the gonads because of the possibility that these may get to the developing embryo.

Among the Protozoa it was found that many that are embryo-symbiotes occur in the arthropods. Some of these such as _Nosema_ _bombycis_, symbiotic in the silk worm embryo, are of great economic importance. There is also a discussion of the physiology of the symbiotic Protozoa. It was found that in the Subclass Phytomastigina only the order Dinoflagellata has members that are symbiotes in embryos or generative organs. In the Zoomastigina there were found more examples of embryo symbiotes. Notable among these are _Histomonas_ _meleagridis_, and the trichomonads which were treated in detail. The totally symbiotic Class Sporozoa has one group, the Microsporidia, many of whose members are symbiotes of embryos. A discussion of the

haemosporidian that causes Texas cattle fever was also included.

Part III devoted to the helminths was divided into sections dealing with the flatworms and the roundworms separately for convenience. Among the trematodes the only embryo symbiotes were found in the digenetic trematodes. The digenetic trematodes found in the bursa Fabricii of birds were included in the discussion. There seem to be no clear cut examples of cestodes as symbiotes of embryos. It was noted that the tissue-cutting nematodes were those most often to be found in embryos where they had wandered from their normal paths through the host's body.

Plant symbiotes of embryonic arthropods are fully discussed. Sections are devoted to extracellular bacteria, intracellular bacterium-like and rickettsia-like forms (including also a discussion of the embryology of the mycetome), rickettsiae, yeasts, fungi, spirochaetes and viruses associated with arthropod embryos and the various methods utilized by both host and symbiote in perpetuating this arrangement. A section is included on the entomophagous insects symbiotic in embryonic araneid and insect eggs and embryos.

The last section of the thesis is a discussion of the author's attempts to infect experimentally chick embryos with the fowl nematode, Heterakis gallinae. There follows a discussion of the methods and procedures used in the experiments, results obtained, and conclusions derived. Two tables which summarize the results are included in the Appendix.

The Appendix comprises 14 tables, twelve of which are host-symbiote lists augmenting the material found under the various topics treated in the thesis.

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